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Screening for Resistance to Sugarcane Brown Rust with Controlled Conditions Inoculation

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SCREENING FOR RESISTANCE TO SUGARCANE BROWN RUST WITH CONTROLLED CONDITIONS INOCULATION

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This thesis is dedicated to my beloved parents Yolanda and Silvio who care for and educate my son Nicolás. Without their inexhaustible love, unconditional support, and dedication, it would be impossible achieve my dreams. Thanks for letting me fly.

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ABSTRACT

Brown rust, caused by *Puccinia melanocephala*, is an important disease of sugarcane. Breeding for host plant resistance is the primary control measure. Screening for resistance has relied on rating the severity of symptoms caused by natural infection; however, erratic results make this method problematic. A method accomplishing both infection and disease expression under controlled conditions could avoid the problems associated with resistance evaluations under natural infection. Inoculation of seedlings was evaluated to determine whether it could provide accurate resistance ratings in cross appraisal, and inoculation under controlled conditions was evaluated for the potential to accurately determine resistance reactions in clones with known and unknown reactions in comparison to field reactions. Seedlings from crosses between parents with different levels of resistance were inoculated with urediniospores at concentrations ranging from 1 x 10^3 to 1 x10^6 spores per ml. Disease severity was visually assessed at 1 and 2 weeks after inoculation, and resistance ratings were assigned on a modified 1 to 9 scale. Inoculum concentration strongly affected severity and the frequency of resistant progeny in crosses. Brown rust resistance is a heritable trait; however, parental reaction was not a consistent determinant of progeny distribution across resistance rating categories. These results suggest that seedling inoculation may not be suitable for the evaluation of brown rust resistance. Clones were inoculated with 1 x10^6 spores per ml, and severity was determined as percentage of leaf area occupied by rust lesions by image analysis. Resistance reactions could not be reliably determined for susceptible clones in single inoculations. Controlled conditions inoculation and natural infection results were not correlated. Multiple inoculations under controlled conditions accurately identified resistant and susceptible clones with severe infection resulting from any single inoculation indicating susceptibility. Therefore, controlled conditions inoculation has the

potential to be useful in limited studies to characterize parents in a recurrent selection program and for basic studies of resistance to brown rust.

CHAPTER 1: GENERAL INTRODUCTION

1.1 BROWN RUST OVERVIEW

Worldwide, sugarcane (inter-specific hybrids of *Saccharum* L.) is a major crop. In 2012, FAO estimated it was cultivated on 23.8 million hectares, in more than 110 countries, with an annual production of 1.77 billion tons of sugarcane stalks (FAOSTAT, 2012). The U.S. occupies 8th place in sugarcane production (27.9 million tons). Brazil is the largest producer with 670 million tons harvested in 2012, followed by India, China and Thailand (FAOSTAT, 2012). In the U.S., sugarcane occupies 5th place after maize, soybean, wheat and sugar beet, with an annual production of more than 27.9 million tons on approximately 350,000 ha (FAOSTAT, 2012). Sugarcane is commercially grown in Florida, Louisiana, Hawaii, and Texas.

Sugarcane production and processing has an important role in the Louisiana economy. The crop is grown on nearly 182,000 ha, and its annual production can exceed 14 million tons of cane (American Sugar Cane League, 2010). The total value to the cane growers and raw sugar factories of the state is more than $800 million with a total economic impact of $2.2 billion. The economic activity generated by this crop provides employment for approximately 17,000 workers in the production and processing of sugar. Louisiana produces about 20% of the sugar produced in the United States (beet and cane).

Brown rust, caused by the fungus *Puccinia melanocephala* Syd. & P. Syd. is an economically important disease in many regions where sugarcane is grown (Ryan and Egan, 1989, Raid and Comstock, 2000). The first report of brown rust in the continental United States was from Florida (Dean *et al*., 1979). Brown rust was observed shortly thereafter in Louisiana (Koike, 1980).

Puccinia melanocephala belongs to the Phylum Basidiomycota, Class Pucciniomycetes, Order Pucciniales, Family Pucciniaceae and the Genus *Puccinia* (Dixon *et al*., 2010). Results of comparative morphology showed that sugarcane rust specimens could be clearly distinguished into two morphologically and phylogenetically distinct groups. The characteristics of the uredinial and telial stages of these two groups corresponded to previously reported taxonomic characteristics of two species, *P. melanocephala* Syd. & P. Syd and *P. kuehnii* E.J. Butler (Virtudazo *et al*., 2001). The life cycle of *P. melanocephala* is simple with the urediniospore being the only known infectious stage.

Appressoria are essential for *Puccinia* spp. to gain entry into the host plants through the stomata. Some exceptions to the typical pattern of stomatal penetration have been reported, especially in rusts of tropical dicotyledonous plants (Sotomayor *et al*., 1983). After the appresorium has developed, a penetration peg, substomatal vesicle, infection hypha, haustorial mother cell, and haustorium are produced in sequence and result in colonization of the host (Sotomayor *et al*., 1983). The development of substomatal vesicles, infectious hyphae, haustoria and subsequent infection processes are similar to other *Puccinia* spp.

The initial symptoms of brown rust are small elongated, yellowish spots, which are visible on both surfaces of the leaf. The elongated spots turn brown to orange-brown or red-brown. The lesions occur irregularly and typically range from 2 to 10 mm in length, but occasionally reach 30 mm. The spots are raised and surrounded by a slight pale yellow halo (Raid and Comstock, 2000).

Red-brown urediniospores are produced in and released from pustules that develop on the underside of leaves (Virtudazo *et al*., 2001). There are abundant capitate paraphyses in uredinia, and urediniospores have dense echinulation with darker brown and uniformly thick walls.

Urediniospore production occurs 8-18 days after the initial urediniospore lands on a leaf, depending on host genotype susceptibility and environmental conditions (Arya *et al*., 2010). Both urediniospores as well as teliospores of *P. melanocephala* have been identified and described (Purdy *et al.*, 1983). Urediniospores are more common and are generally present throughout the season while teliospores are occasionally found towards the end of the season as lesions darken. The dark brown to blackish telia contain brown to dark brown teliospores with apically thickened walls (Virtudazo *et al*. 2001). Basidiospores have been found but do not initiate infection on sugarcane (Purdy *et al.*, 1983).

When the pustules rupture through the lower epidermis, reddish-brown urediniospores are exposed and passively released (Raid and Comstock, 2000). Spread of brown rust occurs primarily by wind dispersal of urediniospores. The movement of diseased vegetative parts of sugarcane, contaminated equipment and workers from one location to another may also provide a means of spread.

On susceptible cultivars, numerous lesions coalesce, causing premature leaf senescence and death. Fields of susceptible cultivars that are heavily infected with brown rust have a reddish-brown tinge visible outside the field (Comstock *et al*., 1992). In general, *P. melanocephala* causes a delay in development of the plant, which manifests itself in reducing the length and final weight of the stalks, and stalk population may be reduced as well (Hoy and Hollier, 2009; Victoria *et al.*, 1984). Brown rust can reduce tonnage yield by 10-20 tons/hectare depending on the length of time brown rust is affecting the cane, but well-timed applications of fungicides can prevent this loss (LSU AgCenter, 2010). In Louisiana, losses in total sucrose up to 22% have been documented (Hoy and Hollier, 2009).

Disease severity is influenced by the interaction of environmental (primarily temperature and leaf wetness), edaphic, genetic and physiological (age of plant) factors. Infection may occur within the temperature range of 5-34^{o}C; however, the optimal temperatures for spore germination are between 15 and 30^{o}C (Barrera, 2010; Barrera *et al*., 2012). Heavy rains tend to remove spores from leaves and the atmosphere, rendering them ineffective if they land on the soil (Comstock and Ferreira, 1986). Several soil factors significantly influence rust infection levels on sugarcane. Studies have shown that rust levels are higher on sugarcane growing on low pH soils and when high soil moisture and high levels of phosphorous, potassium, and sulfur are present in the soil (Anderson *et al*., 1990; Anderson and Dean, 1986; Johnson *et al*., 2007). The disease is more severe in younger plants between 2 and 6 months of age (Raid and Comstock, 2000).

Control of brown rust of sugarcane is largely achieved through the use of resistant cultivars. However, fungicide programs to minimize losses have been developed. Strobilurin fungicides provide the highest level of control (Hoy and Savario, 2007).

1.2 SUGARCANE RESISTANCE TO BROWN RUST

The disease is controlled primarily through the development and cultivation of resistant cultivars (Purdy *et al*., 1983; Raid and Comstock, 2000). The requirement for brown rust resistance places an additional burden on the selection process resulting in the elimination of agronomically promising cultivars; however, breeding has provided control for the disease and has reduced economic losses (Asnaghi *et al.,* 2001; Raid and Comstock, 2000).

Unfortunately, the durability of resistance to brown rust is uncertain, as the pathogen possesses the ability to adapt and overcome host plant resistance. A phenomenon of "boom and

bust" is observed in the behavior of the disease that results in periodic epidemics of sufficient severity to cause significant yield loss. One factor affecting this aspect of the host/pathogen interaction is the extent of cultivation of a resistant cultivar. Extensive cultivation of a single cultivar can create a selection pressure on the pathogen population and lead to a more rapid emergence of a genetic variant of the fungus. Shifts from resistance to susceptibility have been reported in several cultivars in Florida, including CP 78-1247 (Raid *et al*., 1989), CP 79-1580 (Dean and Purdy, 1984), CP 74-2005 and CL 73-239 (Shine *et al.*, 2005). In Louisiana, the cultivar CP 85-384 was ultimately grown on 91% of production area. In 2000, when the LCP 85-384 acreage had increased to over 40%, a severe epidemic of brown rust occurred in what had previously been rated as a resistant cultivar (Hoy and Savario, 2007).

Many resistant cultivars have been identified, but resistance has not been durable on some cultivars (Purdy *et al*., 1983; Raid and Comstock, 2000). Shifts from resistance to susceptibility have been reported for cultivars in different regions (Dean *et al.*, 1984; Hoy and Grisham, 2005; Purdy *et al.*, 1983; Raid, 1989), and these shifts have been suggested to be due to pathogenic specialization. Previous studies evaluating variability in the pathogen population and resistance responses in different host genotypes demonstrated pathogenic variability related to host genotype in the pathogen. Four studies have compared differential reactions resulting from inoculation. In Australia, it was concluded that specialization within the pathogen population to cultivar was not evident (Taylor, 1992), but studies in Florida (Shine *et al.*, 2005) and India (Srinivasan *et al.*, 1965) found differential reactions in cultivars inoculated with pathogen isolates from the same cultivars. In Louisiana, pathogenic specialization to cultivar was detected and quantitative resistance was detected that could be very useful in on-going resistance research

to ultimately improve breeding and selection for effective, durable resistance to brown rust (Avellaneda *et al*., 2013).

Planting a mixture of resistant cultivars is considered one way to reduce the impact of the disease. Varietal diversification may play an important role in holding down the overall area-wide disease pressure, thereby reducing the natural selection pressure for one particular rust pathotype. It is believed that this may assist in preserving the durability of host plant resistance in current resistant cultivars (Raid and Comstock, 2006). The lack of durability in resistance is a very important aspect of brown rust that could be studied with the objective to improve the understanding of expression and basis for resistance in order to develop resistant cultivars with more durable resistance.

Cultivated sugarcane cultivars are complex interspecific aneuploids with chromosome numbers ranging from 2n=80 (Sreenivassan *et al*. 1987). Chu *et al*. (1982) assumed that rust-susceptible genes of modern commercial cultivars are transmitted mainly by some *S. officinarum* clones which account for around 90% of the genome of commercial cultivars, and it has been suggested that resistance was not likely to be determined by a single gene (D'Hont *et al*. 1996). Tai *et al*. (1981) observed marked transgressive segregation towards susceptibility in bi-parental crosses and selfed families and suggested that resistance to rust was partially dominant. Intermediate heritabilities for rust resistance were reported by Tai *et al*. (1981) and Gonzales *et al*. (1987). High narrow-sense and broad-sense heritability values of 0.84 and 0.73 determined by the regression of the progeny mean rust grade on mid-point rust reaction of their parents were reported by Comstock *et al*. (1992), and 0.84 and 0.78 heritability values were reported by Hogarth *et al*. (1993). Daugrois *et al*. (1996) attributed brown rust resistance in the progeny of selfed cultivar R570 to a major resistance gene *Bru*1 with dominant effect. A second major

brown rust resistance gene *Bru*2 (Costet *et al.*, 2012; Raboin *et al*., 2006) was identified and shown to control fungal sporulation. *Bru*1 has been shown to prevent infection by all the rust isolates collected from several geographic origins (Asnaghi *et al.*, 2001).

Natural field infection severity has been the primary means of assessing rust resistance in sugarcane cultivars. Although natural infection is useful in assessing resistance, it is not always reliable in identifying resistant cultivars due to variable environmental conditions and uneven inoculum exposure. Artificial inoculation exposes all plants under disease-favorable conditions to a sufficient urediniospore concentration. Inoculation has been conducted under field conditions by introducing inoculum into the leaf whorl (Sood *et al*. 2009). Inoculation under controlled conditions could provide information about resistance levels in potential parents or seedlings.

The identification of clones resistant to brown rust without relying on natural field infection could help in the breeding program to accurately characterize resistance in potential parents and determine appropriate crosses and thereby obtain a higher frequency of new cultivars resistant to the disease. In addition, the evaluation of resistance under controlled conditions could be of value in phenotyping resistance of mapping populations during the development of molecular markers for resistance. The objectives of this study were to develop brown rust resistance screening methods utilizing inoculation under controlled conditions for clones and seedlings and determine their potential utility for the crossing program and resistance studies.

CHAPTER 2: SEEDLING INOCULATION FOR BROWN RUST RESISTANCE EVALUATION OF SUGARCANE CROSSES

2. 1 INTRODUCTION

Brown rust caused by the biotrophic fungal pathogen *Puccinia melanocephala* Syd. & P. Syd. is an economically important disease of sugarcane (*Saccharum* interspecific hybrids) worldwide. Brown rust symptoms consist of reddish-brown lesions on the leaves, and in severe infections can cause leaf necrosis and premature death of even young leaves (Raid and Comstock 2006). Several variables are associated with disease severity, including host resistance and pathogen genetics (Asnaghi *et al*., 2001; Raid and Comstock, 2000; Shine *et al*., 2005), plant growth stage (Comstock and Ferreira, 1986), weather conditions (Barrera *et al.*, 2013; Irey, 1987; Raid and Comstock, 2006; Sandoval *et al*., 1983), and plant nutrition and soil characteristics (Anderson *et al*., 1990; Anderson and Dean, 1986; Johnson *et al*., 2007). Temperature and leaf wetness are the most important environmental variables affecting brown rust development in susceptible cultivars (Barrera *et al.*, 2013; Purdy *et al*., 1983; Raid and Comstock, 2006; Sandoval *et al*., 1983).

Some cultivars rated as rust resistant develop moderate to severe brown rust while under cultivation. Shifts from resistance to susceptibility have been reported in several cultivars in Florida, including CP 78-1247 (Raid *et al*., 1989), CP 79-1580 (Dean and Purdy, 1984), CP 74-2005 and CL 73-239 (Shine *et al.*, 2005). In Louisiana, what had previously been rated as a resistant cultivar, CP 85-384, developed a severe epidemic of brown rust after commercial acreage had increased to over 40%.

Breeding for host plant resistance has been the primary control measure for brown rust (Raid and Comstock, 2000). Natural infection has been the means of assessing rust resistance in sugarcane clones. Although natural infection is useful in assessing resistance, it is not always

reliable due to variable environmental conditions and uneven inoculum exposure. Resistance to brown rust has been shown repeatedly to be a heritable trait in sugarcane (Comstock *et al.,* 1992, Gonzales *et al.,* 1987, Hogarth *et al.,* 1993, Tai *et al.,* 1981). Therefore, a higher frequency of resistant progeny would be expected to occur in crosses involving resistant parents.

The problems associated with resistance assessment based on natural infection suggest that inoculation methodology should be evaluated for application in breeding programs. Sood *et al*., (2009) evaluated field inoculation by introduction of urediniospores into the leaf whorl to provide uniform exposure of a portion of leaf tissue of all plants under disease favorable conditions and determined that differences in resistance reactions among clones could be detected.

Inoculation under controlled conditions represents another alternative potential method for brown rust resistance screening. It would require inoculum and favorable ambient conditions for infection and symptom development. Barrera *et al.* (2012) demonstrated the feasibility of plant infection and disease expression under controlled conditions and established the conditions favorable for infection. The controlled conditions inoculation method might be useful for determining and comparing brown rust resistance reactions in clonal material or seedlings.

Cross appraisal is a routine part of sugarcane breeding programs to identify agronomically desirable parents and make the most productive crosses. Seedling inoculation might provide an additional component to cross appraisal to identify superior crosses for development of brown rust resistant cultivars. The study objectives were therefore to develop a method for seedling inoculation under controlled conditions and evaluate its potential for cross appraisal of brown rust resistance.

2.2 MATERIALS AND METHODS

2.2.1 Seedling inoculation conditions

Seedlings 4 to 6 weeks of age from crosses between parents with variable brown rust resistance levels were inoculated to compare frequencies of progeny with different resistance ratings assigned following infection and symptom development. In a bi-parental sugarcane cross between two interspecific hybrid parents, each seedling is a unique genotype. In a preliminary experiment, seedlings from five bi-parental crosses were inoculated with *P. melanocephala* urediniospores (Table 2.1).

Table 2.1**.** Crosses inoculated with *Puccinia melanocephala* in a preliminary experiment

Cross	Maternal parent	Paternal parent	Parental reaction[a]
XL 10-069	L 99-226	L 99-233	HS x R
XL 10-139	L 97-128	L 99-226	MS x HS
XL 10-144	L 99-226	L 01-299	HS x R
XL 10-189	HoCP 04-383	LCP 85-384	R x HS
XL 10-197	HoCP 00-950	LCP 85-384	R x HS

[a] Crosses were made between parents rated as having a resistant (R), moderately susceptible (MS) or highly susceptible (HS) reaction to brown rust. Ratings were based on repeated field observations of natural infection severity.

Urediniospores were collected in naturally infected commercial fields with a vacuum sampler from leaves of cultivar Ho 95-988 and stored at -80^{o}C until inoculation. Spore germination rate was determined at the time of collection and each inoculation by plating on water agar and microscopic observation. The preliminary inoculation used urediniospores from a single field with a germination rate of 24.7%. The first inoculation used spores from a second field with a germination rate of 38.9%. The second inoculation used a mixture of spores from the second and a third field with a germination rate of 28.4%.

Seedlings in Styrofoam trays (total of approximately 144 seedlings per cross in two trays each with 72 seedlings) were inoculated by spraying urediniospores at a concentration of 1 x 10^6 per ml in deionized water and 0.1% of surfactant, Tween 20, onto leaves with an atomizer until visibly wet. Spore concentration was adjusted with a haemocytometer. Barrera *et al*., (2012) reported that under controlled conditions, more infection occurred at an optimal infection temperature range of 21-27^{o}C and 10-13 h of leaf wetness. Inoculated trays were placed in an indoor chamber of plastic sheeting, misted with de-ionized water and maintained for a period of 14 hours at 23 ± 1^{o}C (Figure 2.1). Cool mist generators were used to maintain leaf wetness and support spore germination and infection. During the infection period, the temperature was monitored with a thermocouple temperature sensor.

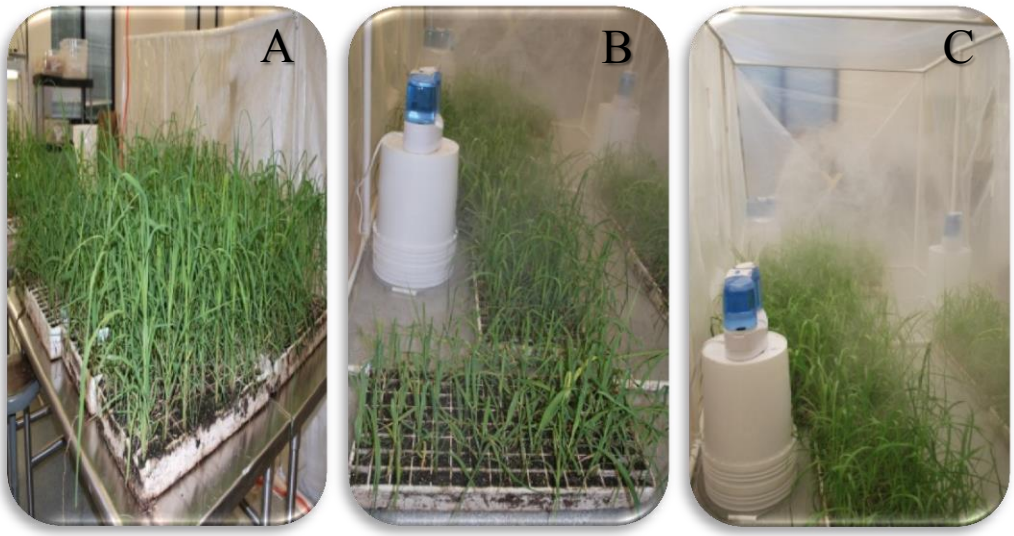

Figure 2.1. Seedling inoculation. A. Seedlings in Styrofoam trays; B. and C. Seedling trays in the mist chamber after inoculation.

After the infection period, plants were placed on shelves at 23^{o}C ±1^o C with a 12 h photoperiod supplied by artificial light (two 40 watt bulbs, light output 2200 lumens) for 2 weeks for symptom development. Individual seedlings were rated for brown rust symptom severity using a modified 1-9 scale (Table 2.2).

Table 2.2. Modified rating scale for brown rust resistance evaluation

Rating	Description
1	**Resistant:** Chlorotic flecks, less than 5 lesions per leaf
3	**Moderately Resistant:** Necrotic flecks, presence of 5-30 lesions per leaf
5	**Moderately Susceptible:** No flecking, more than 30 lesions per leaf
7	**Susceptible:** Some leaves with densely concentrated lesions
9	**Highly Susceptible:** Leaves with high lesion density on most of leaves

Severity ratings were assigned 1 and 2 weeks after inoculation. However, at 1 week, a high percentage of the plants in the preliminary inoculation exhibited severe brown rust symptoms and had died in all five crosses and most plants were dead at 2 weeks. These results suggested that different inoculum concentrations of urediniospores needed to be evaluated.

2.2.2 Effect of inoculum concentration on progeny resistance ratings in bi-parental crosses

Seedlings from crosses between parents with variable resistance levels (Table 2.3) were inoculated to compare frequencies of different severity ratings assigned following inoculation with increasing inoculum concentrations. Four crosses were included in one inoculation (Table 2.3), and seven crosses were included in a second inoculation (Table 2.4). Seedlings within a cross were inoculated with up to four urediniospore concentrations: 1×10^3, 1×10^4, 1×10^5, and 1×10^6 per ml of inoculum. The number of seedlings available after germination for some crosses necessitated use of a reduced number of urediniospore concentrations in each inoculation (Tables 2.5 and 2.6). Separate portions of cross seedling populations were randomly selected for each inoculum concentration. Seedlings in Styrofoam trays were inoculated with each inoculum concentration included and then conditions were provided for infection and symptom development as described previously, except the leaf wetness period in the second inoculation was 10-12 hours instead of 15 hours and the temperature inside the mist chamber was 22.5 ±

2.3°C. Individual seedlings were rated for brown rust symptom severity using the 1 to 9 modified scale (Table 2.1). Ratings were assigned 1 and 2 weeks after inoculation.

Each cross was unique and considered as a separate experiment for analysis. A Proc GLM analysis was conducted after determining the normality of the data using SAS version 9.4 (SAS Institute Inc. Cary, NC, USA) to determine the effects cross, inoculum concentration, weeks after inoculation and possible interactions. The effect of inoculum concentration was determined within each cross with a Chi-square test of independence.

Table 2.3. Crosses included in first inoculation

Cross	Maternal Parent	Paternal parent	Parental reactions[a]
XL 07-065	LCP 81-10	HoCP 96-540	MS x HS
XL 07-082	HoCP 96-540	L 99-226	HS x HS
XL 09-003	LCP 81-10	L 99-233	MS x R
XL 09-100	HoCP 96-540	L 99-233	HS x R

[a] Crosses were made between parents rated as resistant (R), moderately susceptible (MS) or highly susceptible (HS) to brown rust. Ratings were based on repeated field observations of natural field infection severity.

Table 2.4. Crosses included in second inoculation

Cross	Maternal Parent	Paternal parent	Parental reactions[a]
XL 11-062	HoCP 91-552	HoCP 04-838	R x R
XL 11-087	HoCP 04-838	LCP 86-454	R x HS
XL 11-144	L 97-128	L 99-233	MS x R
XL 11-218	LCP 85-384	L 99-226	HS x HS
XL 11-256	HoCP 85-845	L 01-299	MS x R
XL 11-458	Ho 95-988	L 09-125	HS x HS
XL 11-580	Ho 95-988	HoCP 96-540	HS x HS

[a] Crosses were made between parents rated as resistant (R), moderately susceptible (MS) or highly susceptible (HS) to brown rust. Ratings were based on repeated field observations of natural field infection severity.

Table 2.5. Urediniospore concentrations for crosses in the first inoculation

Cross	Urediniospore concentrations for each cross inoculation			
	1.00E+03	1.00E+04	1.00E+05	1.00E+06
XL 07-065	+	+	+	-
XL 07-082	-	+	+	-
XL 09-003	-	+	+	-
XL 09-100	+	+	+	+

Table 2.6. Urediniospore concentrations for crosses in the second inoculation

Cross	Parental reactions[a]	1.00E+03	1.00E+04	1.00E+05	1.00E+06
XL 11-062	R x R	-	+	+	-
XL 11-087	R x HS	+	+	+	+
XL 11-144	MS x R	-	+	+	-
XL 11-218	HS x HS	-	+	+	-
XL 11-256	MS x R	-	+	+	-
XL 11-458	HS x HS	-	+	+	-
XL 11-580	HS x HS	-	+	+	-

2.3 RESULTS

2.3.1 Preliminary inoculation of seedlings from five crosses

One week after inoculation, a high percentage of plants exhibited severe brown rust symptoms and died in all five crosses, and most plants were dead after 2 weeks (Table 2.7). The resistance reactions of the parents had no effect on final mortality.

2.3.2 Effect of inoculum concentration on seedling brown rust severity ratings in bi-parental crosses

Results of disease severity were assessed as the percentage of seedlings assigned to each rating class for each inoculum concentration at 1 and 2 weeks after inoculation. All 11 crosses were unique, so an overall analysis of the results was conducted. The frequency distribution of resistance ratings was affected by cross, inoculum concentration, and week of symptom

assessment (Table 2.8). Significant interactions were detected for all two-way and three-way interactions between cross, inoculum concentration, and time (week) after inoculation (Table 2.8).

Table 2.7. Percentage of seedling mortality in five sugarcane crosses after preliminary inoculation with a urediniospore concentration of 1 x10^6/ml at 1 and 2 weeks after inoculation

Cross	Parental reactions[a]	Week 1 Dead seedlings (%)	Week 2 Dead seedlings (%)
XL 10-069	HS x R	93	98
XL 10-114	HS x R	98	99
XL 10-139	MS x S	81	94
XL 10-197	MS x HS	64	97
XL 10-189	HS x R	95	98

[a] Crosses were made between parents rated as resistant (R), moderately susceptible (MS) or highly susceptible (HS) to brown rust. Ratings were based on repeated field observations of natural field infection severity.

Table 2.8. Fixed effects and interactions for brown rust severity ratings of seedlings in 11 sugarcane crosses resulting from inoculation under controlled conditions with two *Puccinia melanocephala* urediniospore concentrations

Effect	DF	F Value	Pr > F
Cross	10	160.96	<0.0001
Inoculum concentration	1	498.86	<0.0001
Week	1	209.22	<0.0001
Cross*Inoculum	10	29.75	<0.0001
Cross*Week	10	6.95	<0.0001
Inoculum*Week	1	6.65	0.0099
Cross*Inoculum*Week	10	2.63	0.0034

The frequency distribution of seedlings assigned different severity ratings was affected by inoculum concentration and time after inoculation in different crosses, so the results are presented separately for each cross at 1 and 2 weeks after inoculation. The number of seedlings available resulted in different inoculum concentrations being evaluated. Cross XL 07-065 (MS x HS) was inoculated with three different urediniospores concentrations (Figure 2.2). A high

percentage of resistant seedlings was observed when seedlings were inoculated with a concentration of 1 x 10^3spores/ml, and no seedlings were observed with susceptible ratings In contrast, inoculation with a concentration of 1 x 10^5 spores/ml resulted in a higher percentage of susceptible seedlings. Although, no seedlings received a rating of 9. Chi-square analysis indicated that the results of this cross were influenced by inoculum concentration at both 1 and 2 weeks after inoculation (X^2=167.2; P= <0.0001 for week 1 and X^2=122.8; P= <0.0001for week 2) (Tables 2.9 and 2.10).

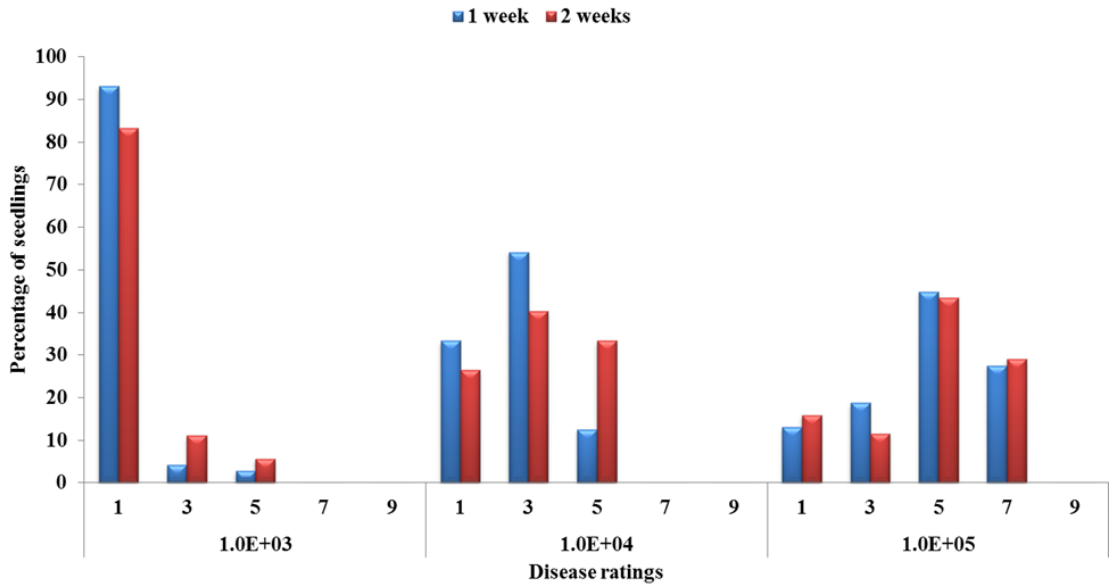

Figure 2.2. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 07-065 (moderately susceptible x highly susceptible parents) 1 and 2 weeks after inoculation with three concentrations of urediniospores of *Puccinia melanocephala*.

Table 2.9. Frequency distribution of seedlings across severity ratings for cross XL 07-065 inoculated with three urediniospore concentrations and rated 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+03	62	4	1	0	0	67
1.0E+04	22	39	9	0	0	70
1.0E+05	5	13	31	19	0	68

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.10. Frequency distribution of seedlings across severity ratings for cross XL 07-065 inoculated with three urediniospore concentrations and rated 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+03	27	8	5	0	28	67
1.0E+04	7	29	24	0	10	70
1.0E+05	4	8	30	20	6	68

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Cross XL 07-082 was inoculated with only two inoculum concentrations (Figure 2.3). At the lower 1 x 10^4 inoculum concentration, there wase a high percentage of resistant seedlings. However, increasing the inoculum concentration resulted in more susceptible seedlings. Inoculum concentration affected the frequency distribution of seedlings across severity ratings (X^2=68.4; P= <0.0001 for week 1 and X^2=55.4; P= <0.0001for week 2) (Tables 2.11 and 2.12).

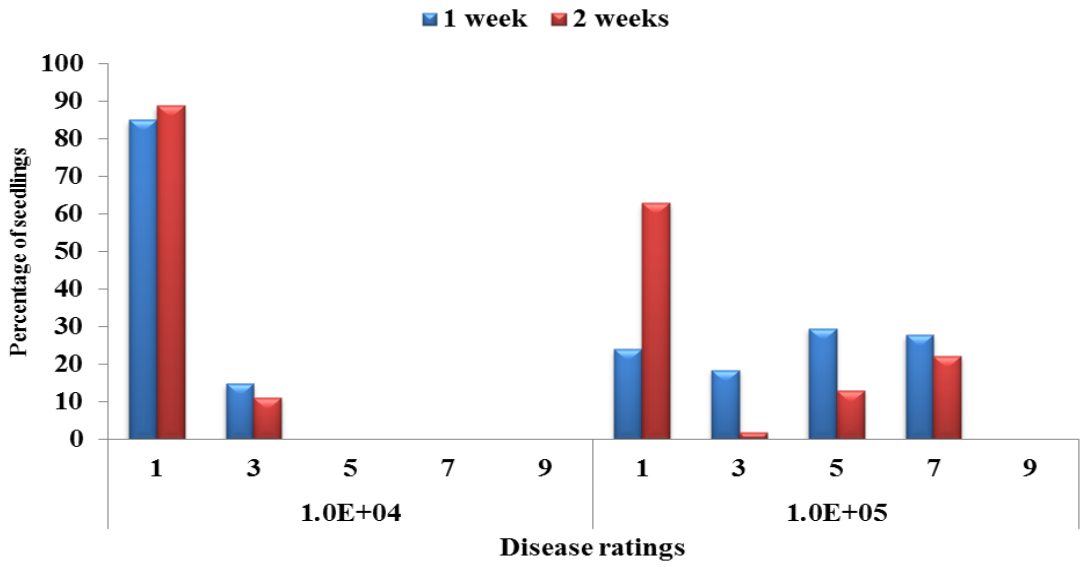

Figure 2.3.Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 07-082 (highly susceptible x highly susceptible parents) 1 and 2 weeks after inoculation with two concentrations of urediniospores of *Puccinia melanocephala.*

Table 2.11**.** Frequency distribution of seedlings across severity ratings for cross XL 07-082 inoculated with two urediniospore concentrations and rated 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	43	8	0	0	0	51
1.0E+05	2	10	16	15	0	43

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.12**.** Frequency distribution of seedlings across severity ratings for cross XL 07-082 inoculated with two urediniospore concentrations and rated 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	33	6	0	0	12	51
1.0E+05	1	1	7	12	22	43

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Cross XL 09-003 (MS x R) showed a frequency distribution of seedlings across resistant and susceptible severity ratings for two inoculum concentrations (Figure 2.4). Inoculum concentration did not affect the frequency distribution of the seedlings across severity ratings at both 1 and 2 weeks after inoculation (X^2=5.1; P= 0.1681 for week 1 and X^2=18.6608; P= <0.0001for week 2) (Tables 2.13 and 2.14).

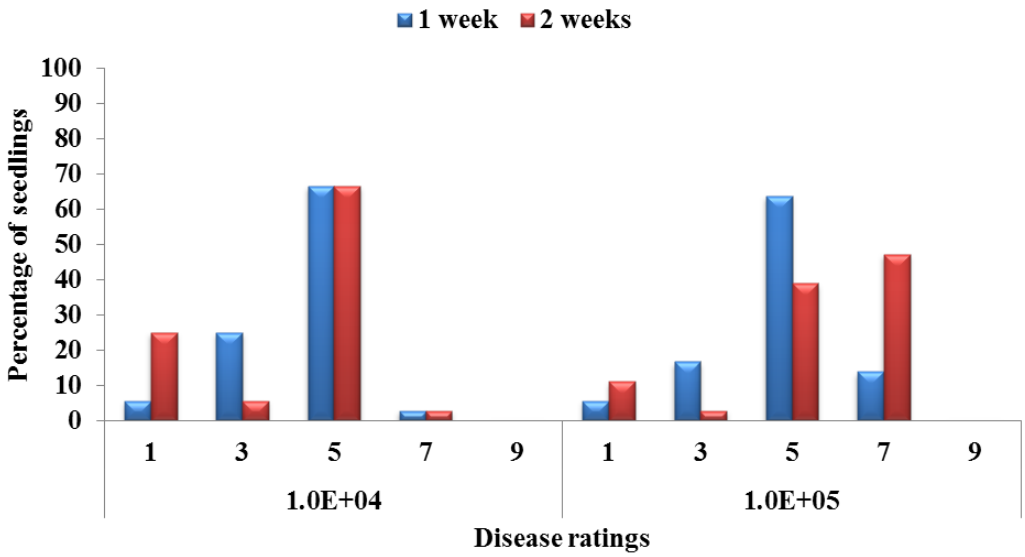

Figure 2.4.Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9= highly susceptible) in cross XL 09-003 (moderately susceptible x resistant parents) 1 and 2 weeks after inoculation with two concentrations of urediniospores of Puccinia melanocephala.

Table 2.13. Frequency distribution of seedlings across severity ratings for cross XL 09-003 inoculated with two urediniospore concentrations and rated for severity 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	0	7	27	1	0	35
1.0E+05	2	6	23	5	0	36

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.14. Frequency distribution of seedlings across severity ratings for cross XL 09-003 inoculated with two urediniospore concentrations and rated for severity 2 weeks after inoculation

Urediniospore concentration	Severity rating[a]					
	1	3	5	7	9	Total
1.0E+04	0	1	25	1	8	35
1.0E+05	1	1	14	16	4	36

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Cross XL 09-100 (HS x R) exhibited variable frequency distributions of seedlings across severity ratings for four inoculum concentrations with increased frequencies in susceptible ratings at higher concentrations (Figure 2.5). Inoculum concentration affected the frequency distribution of seedlings across severity ratings in both weeks (X^2=494.8; P= <0.0001 for week 1 and X^2=330.42; P= <0.0001for week 2) (Tables 2.15 and 2.16).

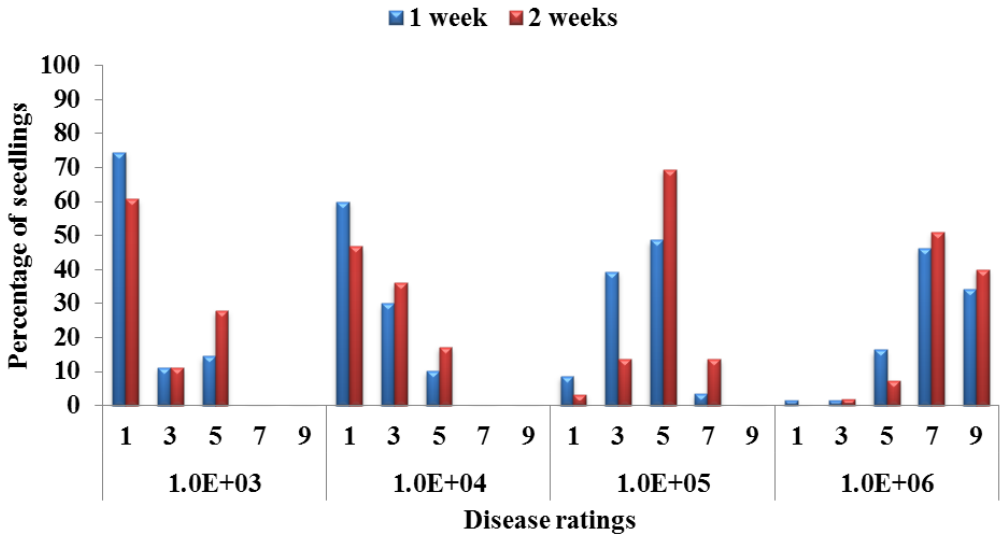

Figure 2.5. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 09-100 (highly susceptible x resistant parents) 1 and 2 weeks after inoculation with four concentrations of urediniospores of *Puccinia melanocephala*.

Table 2.15. Frequency distribution of seedlings across severity ratings for cross XL 09-100 inoculated with three urediniospores concentrations and rated for severity at 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+03	95	14	19	0	0	128
1.0E+04	82	41	14	0	0	137
1.0E+05	12	56	67	5	0	140
1.0E+06	1	1	11	31	23	67

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.16. Frequency distribution of seedlings across severity ratings for cross XL 09-100 inoculated with three urediniospores concentrations and rated for severity 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+03	65	12	31	0	20	128
1.0E+04	57	44	21	0	15	137
1.0E+05	4	19	84	17	16	140
1.0E+06	0	1	5	28	33	67

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Seedlings from XL 11-062, the only cross between two resistant parents, exhibited high frequencies of seedlings in the resistant severity ratings at two inoculum concentrations, 1×10^4 and 1×10^5spores/ml, with seedlings only in the two resistant severity ratings when inoculated with 1×10^4 spores/ml (Figure 2.6). However, Chi-square analysis indicated that the frequency distribution of seedlings was affected by inoculum concentration at both times after inoculation (X^2=33.5; P= <0.0001 for week 1 and X^2=66.1; P= <0.0001for week 2) (Tables 2.17 and 2.18).

Table 2.17. Frequency distribution of seedlings across severity ratings for cross XL 11-062 inoculated with two urediniospore concentrations and rated for severity 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	101	16	0	0	0	117
1.0E+05	63	49	8	0	0	120

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

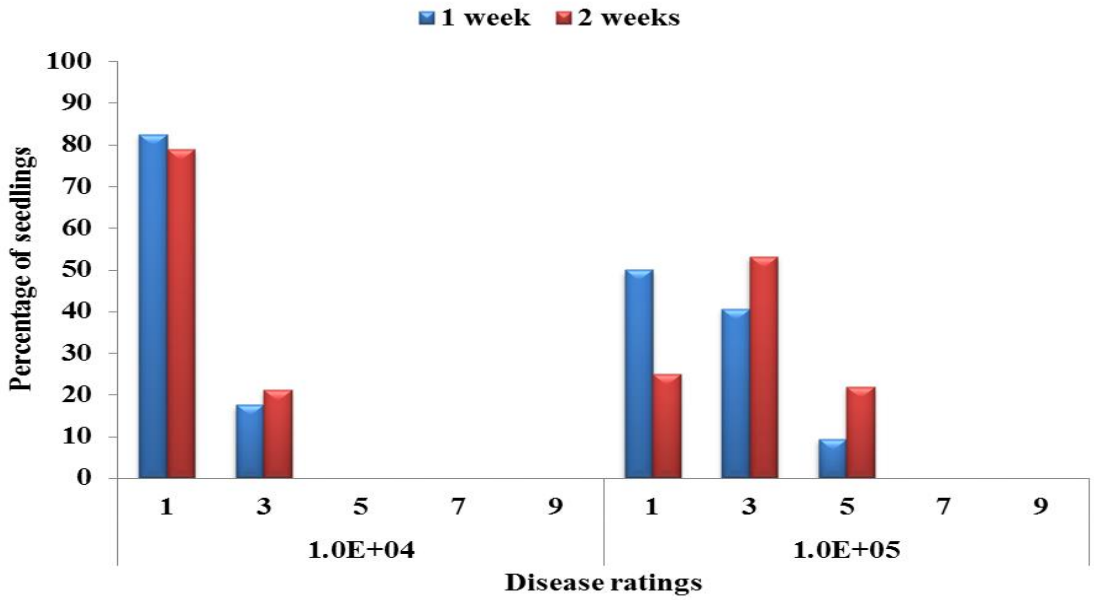

Figure 2.6. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 11-062 (resistant x resistant parents) 1 and 2 weeks after inoculation with two concentrations of urediniospores of *Puccinia melanocephala*.

Table 2.18. Frequency distribution of seedlings across severity ratings for cross XL 11-062 inoculated with two urediniospore concentrations and rated for severity 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	98	19	0	0	0	117
1.0E+05	40	58	20	0	2	120

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Cross XL 11-087 (R x HS) inoculated with two inoculum concentrations exhibited a distribution of seedlings across more severity ratings; however, the frequency distribution was affected by inoculum concentration (X^2=48.1; P= <0.0001 for week 1 and X^2=43.3; P= <0.0001for week 2) (Figure 2.7, Tables 2.19 and 2.20). As the inoculum concentration increased, the distribution shifted to more susceptible ratings. However, seedlings with highly susceptible ratings were not recorded even at the highest inoculum concentration.

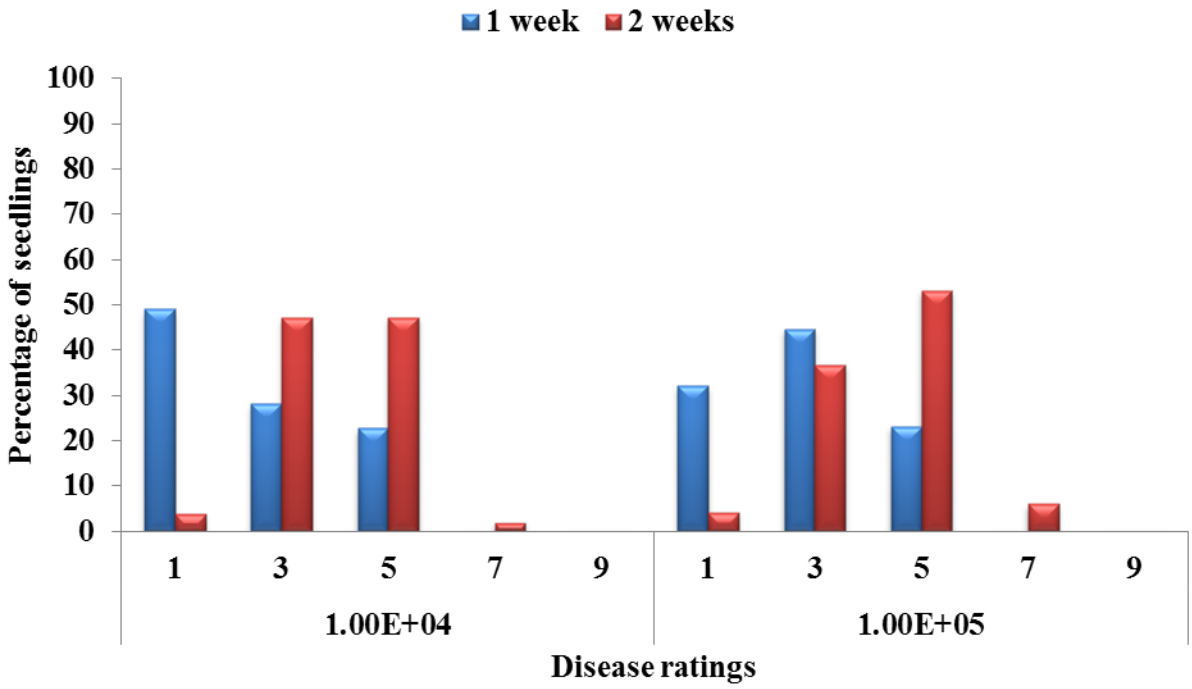

Figure 2.7. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 11-087 (resistant x highly susceptible parents) 1 and 2 weeks after inoculation with four concentrations of urediniospores of *Puccinia melanocephala*.

Table 2.19. Frequency distribution of seedlings across severity ratings for cross XL 11-087 inoculated with three urediniospores concentrations and rated for severity at 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+03	29	17	9	0	0	55
1.0E+04	28	16	13	0	0	57
1.0E+05	11	28	14	2	0	55
1.0E+06	2	25	25	1	0	53

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating..

Table 2.20. Frequency distribution of seedlings across severity ratings for cross XL 11-087 inoculated with three urediniospores concentrations and rated for severity at 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+03	23	19	13	0	0	55
1.0E+04	18	25	13	0	1	57
1.0E+05	6	26	18	2	3	55
1.0E+06	2	18	26	3	4	53

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Seedlings from two MS x R crosses, XL 11-144 and XL 11-256, showed frequency distributions of seedlings mostly with resistant severity ratings for both inoculum concentrations and times after inoculation (Figures 2.8 and 2.9). Chi square analysis indicated that the frequency distributions across severity ratings were similar for both inoculum concentrations at 1 week after inoculation for both crosses (X^2=0.1; P= 0.7 for XL 11-144 and X^2=3.6; P= 0.1626 for XL 11-256). However, the seedling frequency distributions were affected by inoculum concentration for both crosses at 2 weeks after inoculation (X^2=5.0; P= 0.0251for XL 11-144 X^2=21.5; P= <0.0001for XL 11-256) (Tables 2.21, 2.22, 2.23 and 2.24).

Three crosses between highly susceptible parents, XL 11-218, XL 11-458 and XL 11-580, showed variable distributions of seedlings across severity ratings with two inoculum concentrations, 1 x 10^4 and 1 x 10^5spores/ml (Figures 2.10, 2.11, and 2.12 and Tables 2.25, 2.26, 2.27, 2.28, 2.29. and 2.30, respectively). Seedlings of crosses XL 11-218 and XL 11-580 were distributed mostly in the resistant severity ratings. The distribution of seedlings from cross XL 11-458 extended to the susceptible severity ratings, particularly with an inoculum concentration of 1 x 10^5spores/ml. The distribution of seedlings across ratings was affected by inoculum concentration for either time after inoculation for all three crosses (X^2=12.5; P= 0.0019 for week 1 and X^2=38.1; P= <0.0001for week 2 for XL 11-218; X^2=142.7; P= <0.0001 for week 1 and X^2=151.3; P= <0.0001 for week 2 for XL 11-458; and X^2=79.4; P= <0.0001 for week 1 and X^2=112.0; P= <0.0001 for week 2 for XL 11-580).

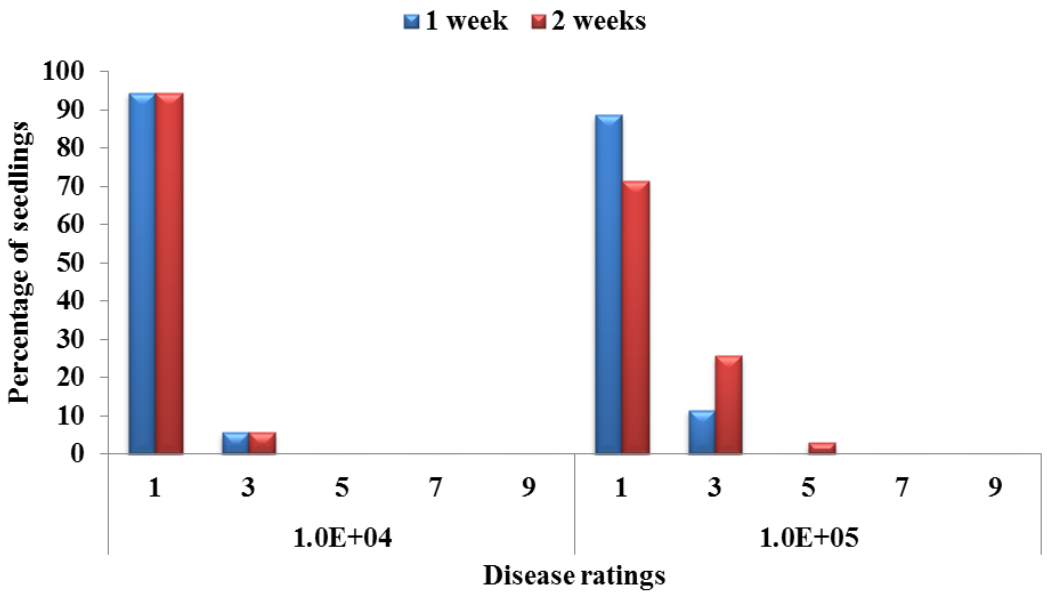

Figure 2.8. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 11-144 (moderately susceptible x resistant parents) 1 and 2 weeks after inoculation with two concentrations of urediniospores of *Puccinia melanocephala*.

Table 2.21. Frequency distribution of seedlings across severity ratings for cross XL 11-144 inoculated with two urediniospore concentrations and rated for severity 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	66	5	0	0	0	71
1.0E+05	65	6	0	0	0	71

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.22. Frequency distribution of seedlings across severity ratings for cross XL 11-144 inoculated with two urediniospore concentrations and rated for severity 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	64	7	0	0	0	71
1.0E+05	54	17	0	0	0	71

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

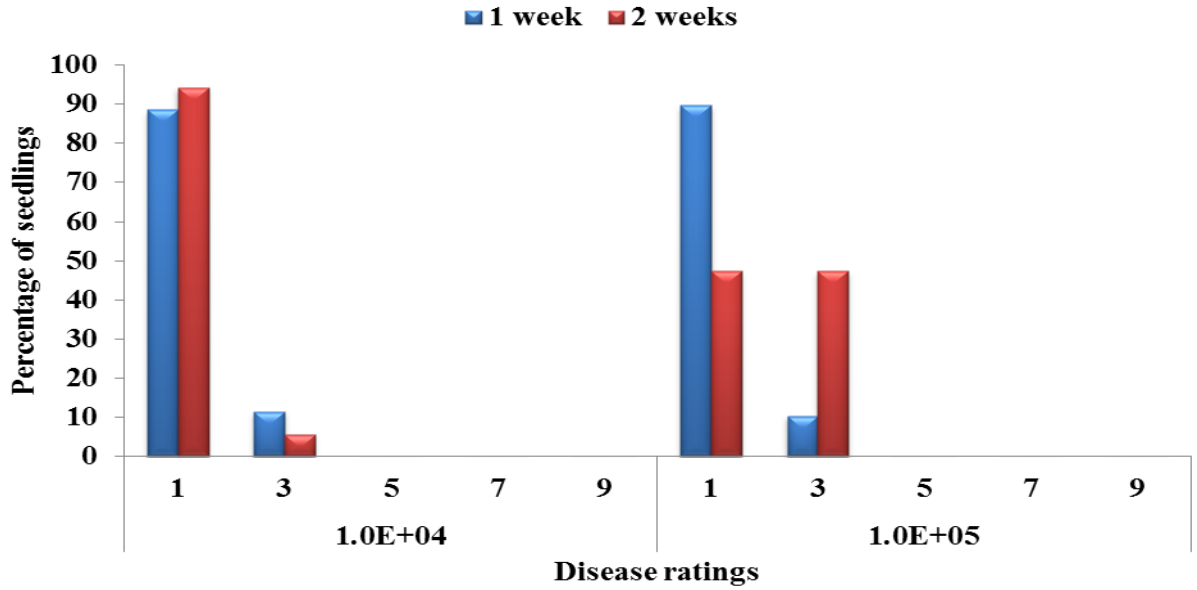

Figure 2.9. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 11-256 (moderately susceptible x resistant parents) 1 and 2 weeks after inoculation with two concentrations of urediniospores of *Puccinia melanocephala*.

Table 2.23. Frequency distribution of seedlings across severity ratings for cross XL 11-256 inoculated with two urediniospore concentrations and rated for severity 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	66	10	0	0	0	76
1.0E+05	57	15	2	0	0	74

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.24. Frequency distribution of seedlings across severity ratings for cross XL 11-256 inoculated with two urediniospore concentrations and rated for severity 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	55	20	0	0	1	76
1.0E+05	27	40	5	0	2	74

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

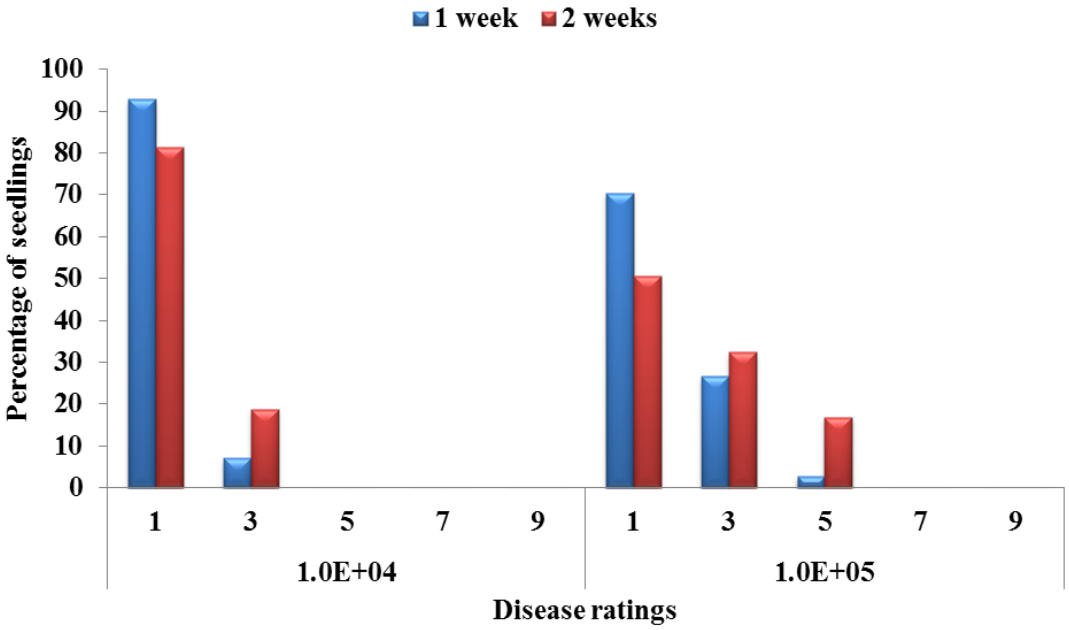

Figure 2.10. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 11-218 (highly susceptible x highly susceptible parents) 1 and 2 weeks after inoculation with two concentrations of urediniospores of *Puccinia melanocephala.*

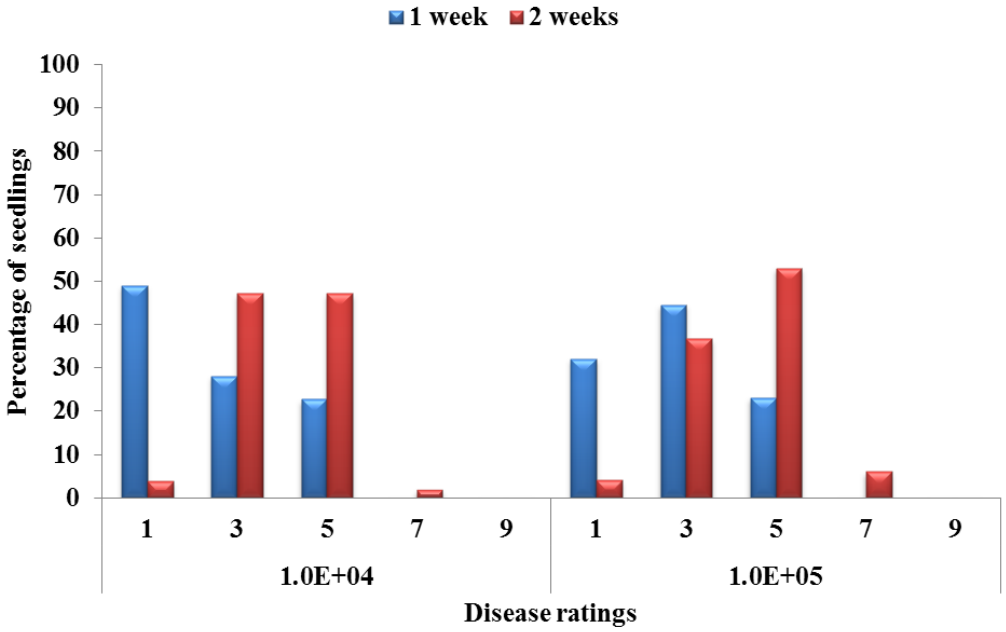

Figure 2.11. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 11-458 (highly susceptible x highly susceptible parents) 1 and 2 weeks after inoculation with two concentrations of urediniospores of *Puccinia melanocephala*.

Table 2.25. Frequency distribution of seedlings across severity ratings for cross XL 11-218 inoculated with two urediniospore concentrations and rated for severity 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	124	14	0	0	0	138
1.0E+05	105	35	2	0	0	142

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.26. Frequency distribution of seedlings across severity ratings for cross XL 11-218 inoculated with two urediniospore concentrations and rated for severity 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	106	31	0	0	1	138
1.0E+05	67	52	23	0	0	142

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.27. Frequency distribution of seedlings across severity ratings for cross XL 11-458 inoculated with two urediniospore concentrations and rated for severity 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	117	24	2	0	0	143
1.0E+05	16	69	48	2	0	135

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.28. Frequency distribution of seedlings across severity ratings for cross XL 11-458 inoculated with two urediniospore concentrations and rated for severity 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	81	58	4	0	0	143
1.0E+05	4	41	84	5	1	135

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

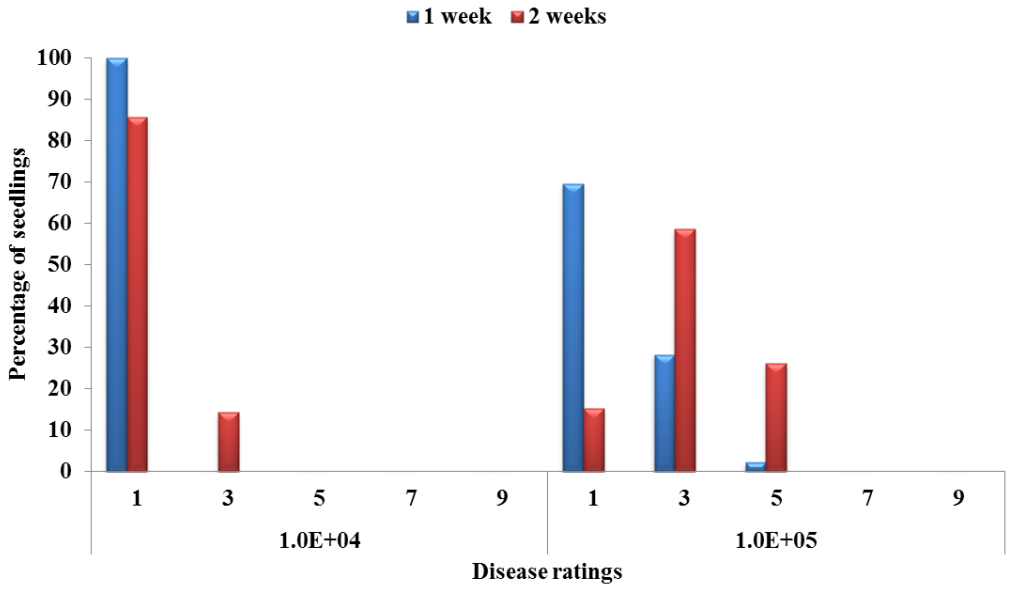

Figure 2.12. Frequencies of disease severity ratings assessed on a 1-9 scale (1=highly resistant and 9=highly susceptible) for seedlings from cross XL 11-580 (highly susceptible x highly susceptible parents) 1 and 2 weeks after inoculation with two concentrations of urediniospores of *Puccinia melanocephala*.

Table 2.29. Frequency distribution of seedlings across severity ratings for cross XL 11-580 inoculated with two urediniospore concentrations and rated for severity 1 week after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	94	9	0	0	0	103
1.0E+05	37	51	11	1	0	100

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

Table 2.30. Frequency distribution of seedlings across severity ratings for cross XL 11-580 inoculated with two urediniospore concentrations and rated for severity 2 weeks after inoculation

	Severity rating[a]					
Urediniospore concentration	**1**	**3**	**5**	**7**	**9**	**Total**
1.0E+04	79	24	0	0	0	103
1.0E+05	7	51	41	1	0	100

[a] Severity ratings were assigned on a modified 1-9 scale in which 1=highly resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, and 9=highly susceptible. Values in columns are number of seedlings assigned with each severity rating.

To evaluate the possible relationship between the frequency of resistant progeny with parental resistance reactions, the percentages of seedlings with resistant severity ratings of 1 and 3 were combined for each cross and compared for inoculum concentrations of 1×10^4 and 1×10^5 spores/ml (the inoculum concentrations that produced the widest distribution of seedlings across ratings) at 1 and 2 weeks after inoculation (Table 2.32). Inoculation with the lower spore concentration resulted in progeny frequency distributions strongly skewed toward the resistant ratings, particularly at 1 week after inoculation. Three crosses, XL 07-065 (MS x HS), XL 07-082 (HS x HS), and XL 09-100 (HS x R), exhibited a strong shift to higher frequencies of seedlings with susceptible ratings when inoculated with the higher spore concentration while the other crosses did not.

To further compare a possible association between parental resistance and the frequency of resistant progeny in a cross, the total frequencies of seedlings with resistant ratings were compared for each cross type using the more severe inoculation (1 x 10^5 spores/ml at 2 weeks after inoculation) (Table 2.31).The frequency of resistant seedlings in six crosses with at least one resistant parent ranged from 3.1 to 97.1% (55.1% average). In comparison, the frequency of resistant seedlings in five crosses with susceptible parents ranged from 9.6 to 94.8% (57.8% average). High percentages of resistant seedlings were observed for three crosses between highly susceptible parents, XL 11-218, XL 11-087 and XL 11-580, even with the more severe inoculation conditions.

Table 2.31. Percentage of resistant seedlings from 11 sugarcane crosses rated at 1 and 2 weeks after inoculation with two Puccinia melanocephala urediniospore concentrations

Cross	Parental reactions[a]	Urediniospore concentration			
		1.0E+04*		1.0E+05*	
		1 week (%)	2 weeks (%)	1 week (%)	2 weeks (%)
XL 07-065	MS x HS	87.5	66.7	31.8	27.5
XL 07-082	HS x HS	100	100	28.0	9.6
XL 09-003	MS x R	26.5	7.4	22.3	3.1
XL 09-100	HS x R	89.8	82.8	47.9	16.9
XL 11-062	R x R	100	100	90.6	78.1
XL 11-087	R x HS	100	100	100	94.8
XL 11-144	MS x R	100	100	100	97.1
XL 11-218	HS x HS	100	100	97.2	83.1
XL 11-256	MS x R	100	100	100	94.8
XL 11-458	HS x HS	77.2	51	76.7	40.8
XL 11-580	HS x HS	100	100	97.9	73.9

[a]Crosses were made between parents rated as resistant (R), moderately susceptible (MS) or highly susceptible (HS) to brown rust. Parent ratings were based on repeated field observations of natural infection severity.

Table 2.32. Percentage of resistant seedlings from 11 crosses with different parental resistance reactions to brown rust

Parental reactions[a]					
R x R	**MS x R**	**R x HS**	**HS x R**	**MS x HS**	**HS x HS**
78.1	3.1	40.8	16.9	27.5	9.6
	97.1				83.1
	94.8				94.8
					73.9

[a]Crosses were made between parents rated as resistant (R), moderately susceptible (MS) or highly susceptible (HS) to brown rust. Parent ratings were based on repeated field observations of natural infection severity. Values are percentages of seedlings rated as resistant from 11 different crosses determined at 2 weeks after inoculation with *Puccinia melanocephala* urediniospores at an inoculum concentration of 1 x 10^5 spores/ml.

2.4 DISCUSSION

The study results demonstrated that the concentration of *Puccinia melanocephala* urediniospores affects the severity of infection of sugarcane seedlings following inoculation under controlled conditions. This in turn affects the frequency of resistant progeny recorded in bi-parental crosses. Apparently, inoculation with a high inoculum concentration under highly favorable conditions for infection can overcome brown rust resistance in seedlings.

Ratings of seedlings also were affected by the time after inoculation for assessment. Severity and the frequency of seedlings with susceptible ratings generally increased between 1 and 2 weeks after inoculation. The higher severity detected at 2 weeks after infection is consistent with the brown rust latent period reported in previous studies. Sotomayor *et al.* (1983) reported the rupture of epidermis and formation of urediniospores beginning 7 days after inoculation and Irey (1987) described a time period between 8 and 11 days to produce a new generation of spores.

Evaluation of resistance using inoculation under controlled conditions as a part of cross appraisal in the breeding program would involve comparisons between crosses. This would be facilitated by a potential distribution of seedling ratings across the full scale of resistance

categories. Inoculation with the lowest (1 x 10^3 spores/ml) and highest (1 x 10^6 spores/ml) inoculum concentrations resulted in seedling frequency distributions that were skewed toward resistant or susceptible ratings, respectively. Inoculation with 1 x 10^6 spores/ml overwhelmed resistance. Inoculation with 1 x 10^4 and 1 x 10^5 spores/ml more often resulted in a wider distribution of ratings. At these inoculum concentrations, differences in the frequency of resistant progeny could be detected among crosses. However, an optimal combination of inoculum concentration and assessment time after inoculation for comparing crosses was not obvious. Disease severity was lower in the second inoculation perhaps due to a slightly shorter (2-3 h) leaf wetness period (urediniospore germination was lower also in the second inoculation). An increase in disease severity with increasing leaf wetness period was demonstrated previously for brown rust (Barrera *et al.*, 2012); however, leaf wetness periods between 10 and 13 hours were favorable for infection. The frequency of seedlings rated as resistant to brown rust was affected by both inoculum concentration and environmental conditions regardless of the resistance reactions of the parents being crossed.

Resistance to brown rust has been shown repeatedly to be a heritable trait in sugarcane (Comstock *et al.*, 1992, Gonzales *et al.*, 1987, Hogarth *et al.*, 1993, Tai *et al.*, 1981). Therefore, a higher frequency of resistant progeny would be expected to occur in crosses involving resistant parents. The inoculation of seedling populations from bi-parental crosses under controlled conditions did not consistently produce this expected outcome. Comparing infection severity assessed as ratings in the progeny of 11 crosses, parental reaction was not a reliable determinant of the frequency of resistant offspring. Crosses between highly susceptible parents showed a high percentage of resistant seedlings in three of four crosses 2 weeks after inoculation with 1 x 10^5 spores/ml.

The mechanisms of resistance to brown rust are still being elucidated. A major brown rust resistance gene, *Bru*1, with dominant effect was reported (Daugrois *et al.*, 1996). This was followed by the identification of a second resistance gene, *Bru*2 (Costet *et al*., 2012; Raboin *et al*., 2006). *Bru*1 has been shown to prevent infection by diverse rust isolates collected from widespread geographic origins and has thus far provided a high level of durable resistance (Asnaghi *et al.*, 2001). *Bru*1 is widely distributed with variable frequency in the breeding population and cultivars in different areas (Costet *et al.*, 2012, Glynn *et al*., 2013). This gene occurs at low frequency in Louisiana sugarcane germplasm (Parco *et al.*, 2014). It is present in only one commercial cultivar, L 01-299. This cultivar was a parent in one of the crosses in this study, and there was a high frequency of resistant seedlings (95%) following inoculation. Unfortunately, this cross was not inoculated with the highest inoculum concentration to evaluate how resistant phenotype frequency would be affected by high inoculum pressure in the presence of *Bru*1 in the seedling population. A second resistant cultivar, L 99-233, included in three crosses in the study, was previously demonstrated to exhibit quantitative resistance to brown rust (Avellaneda *et al.*, 2013), and seedlings in crosses with L 99-233 as a parent showed a strong effect of inoculum concentration on the frequency of a resistant phenotype in two of three crosses.

Parental reaction was not a consistent determinant of offspring distribution across resistance categories in this study. Therefore, seedling inoculation under controlled conditions to evaluate brown rust resistance will not be useful as a part of cross appraisal in the sugarcane breeding program. Transplant of surviving (potentially resistant) seedlings to the field resulted in poor survival (J. W. Hoy, unpublished). Resistance mechanisms to brown rust are either not fully expressed or can be overwhelmed in seedlings under conditions highly favorable for disease. The

relevance of this finding for understanding the genetics and expression of resistance warrants further investigation. The results suggest seedlings are not a good stage for identification of resistance to brown rust in a cultivar selection program.

CHAPTER 3: COMPARISON OF RESISTANCE SCREENING METHODS FOR BROWN RUST OF SUGARCANE BASED ON CONTROLLED CONDITIONS INOCULATION AND NATURAL FIELD INFECTION

3.1 INTRODUCTION

Brown rust of sugarcane (*Saccharum* interspecific hybrids), caused by *Puccinia melanocephala* Syd. and P. Syd., is an important disease worldwide that can cause yield losses greater than 50% (Purdy *et al*., 1983, Raid and Comstock, 2000). In Louisiana, total sucrose yield losses up to 22% were reported from a susceptible cultivar, LCP 85-384 (Hoy and Hollier, 2009). Effective brown rust management is needed for successful sugarcane production.

The use of resistant cultivars is the preferred method of control for brown rust, and screening for resistance is incorporated into sugarcane breeding programs (Purdy *et al*., 1983; Raid and Comstock, 2000). The requirement for brown rust resistance places an additional burden on the selection process resulting in the elimination of agronomically promising clones (Raid and Comstock, 2000); however, host plant resistance is a desirable management option because it can effectively control disease without any direct cost to the growers. Unfortunately, the adaptability of *P. melanocephala* can adversely affect the durability of resistance.

Shifts from resistance to susceptibility have been reported repeatedly for commercial cultivars (Dean and Purdy, 1984; Hoy and Savario, 2007; Raid *et al*., 1989; Shine *et al.*, 2005), and differential interactions between cultivars and pathogen populations have been demonstrated (Avellaneda *et al.*, 2013; Shine *et al.*, 2005; Srinivasan *et al.*, 1965). Resistance to brown rust is a heritable trait with quantitative expression (Comstock *et al*., 1992; D'Hont *et al*. 1996; Gonzales *et al*., 1987; Hogarth *et al*., 1993; Tai *et al*., 1981). Two major resistance genes, *Bru*1 and *Bru*2, have been identified (Costet *et al.*, 2012; Daugrois *et al*., 1996; Raboin *et al*., 2006), and *Bru*1 was demonstrated to prevent infection by rust isolates from multiple geographic areas (Asnaghi

et al., 2001). In Louisiana, *Bru*1 was detected in only one cultivar, L 01-299 (Parco *et al.*, 2014), and quantitative resistance was demonstrated in cultivar L 99-233 (Avellaneda *et al.*, 2013).

The evaluation of resistance to brown rust has relied almost exclusively on observation and rating of natural infection severity in the field (Asnaghi *et* al., 2001; Raid and Comstock, 2000, Tai *et al*., 1981). This approach is problematic because of erratic clone resistance reactions due to variable environmental conditions and inoculum exposure. A resistance screening method utilizing inoculation of the emerging leaf whorl under field conditions was developed to provide a more controlled comparison of clone reactions (Sood *et al*., 2009). However, this method also can be affected by variation in plant phenotype and environmental conditions (Hoy unpublished).

A method accomplishing both infection and disease expression under controlled conditions could avoid the problems associated with resistance evaluations under natural infection. This might allow for more reliable rating of the brown rust resistance reactions of clones of interest in a recurrent selection program and basic studies of the nature and expression of resistance. The objective of this study was to evaluate screening under controlled conditions favorable for infection in comparison to natural field infection in a population of clones with known and unknown resistance reactions to determine its suitability for evaluating brown rust resistance.

3.2 MATERIALS AND METHODS

3.2.1 Clones and inoculation methods

Plants of 21 sugarcane clones were produced vegetatively from single-node cuttings in the greenhouse and used in three experiments to evaluate the ability of inoculation under controlled conditions to determine and compare brown rust resistance ratings in clones with variable levels of resistance (Table 3.1). The study included commercial cultivars with known

resistance reactions and unreleased clones with unknown reactions. Four susceptible cultivars, LCP 85-384, Ho 95-988, HoCP 96-540, and L 99-226, and two resistant cultivars, L 99-233 and L 01-299, were included. The plants were grown in 3.8 liter pots in a 1:1 mixture of silt loam soil and sand and had approximately four fully emerged leaves at the time of inoculation.

Urediniospores of *P. melanocephala* were collected by vacuum from the abaxial surface of multiple naturally infected leaves in naturally infected fields of cultivar Ho 95-988 and stored at -80 ^{o}C. Plants were inoculated with a concentration of 1 x 10^{6} urediniospores/ml in deionized water with 0.1% Tween 20 surfactant. Spore concentration was assessed and adjusted with a haemocytometer. Spore germination rate was determined at the time of each inoculation by plating on water agar and microscopic observation, except for the first inoculation. The first inoculation used spores from a single field and the germination rate was 31.2%. The second and third inoculations were done with spores from a second field and the germination rates were 38.1% and 29.2%, respectively. Inoculum was applied to both sides of two fully emerged leaves per plant with a brush until a film of moisture was visible (Barrera *et al*. 2012). Three plants of each cultivar were inoculated and placed in an indoor chamber of plastic sheeting, misted with distilled water, and kept in the chamber for 15 hours. Cool mist generators (Kaz Incorporated, Hudson, NY,USA)were used to maintain leaf wetness. A temperature of 23 ± 1 ^{o}C was maintained to promote spore germination and infection (Barrera *et al.,* 2012). After the infection period, the plants were relocated to shelves under artificial lighting (two 40 watt bulbs, 2200 lumens each) at room temperature with a photoperiod of 12 h/day.

After 2 weeks, inoculated leaves were cut, scanned, and the percentage of leaf area occupied by brown rust lesions was determined by image analysis using Assess 2.0 Image Analysis Software (APS Press, American Phytopathological Society, St. Paul, MN, U.S.).

Resistance ratings were assigned to clones using a 1-9 scale in which 1 to 3 are resistant categories, 4 to 6 are moderately susceptible categories, and 7 to 9 are highly susceptible categories. Disease severity for the commercial cultivars known to be highly susceptible to brown rust provided the basis for assignment of severity ratings. The leaf infection percentages for three highly susceptible cultivars, LCP 85-384, Ho 95-988, and HoCP 96-540, were averaged, and that value was assigned a rating of 7. That percentage was then divided by seven to determine the rating percentage intervals and assign ratings to all clones.

After testing the normality of the data by Shapiro-Wilk, statistical analysis of the percent leaf area occupied by lesions data was performed using PROC GLM from SAS Version 9.4 (SAS Institute Inc. Cary, NC, USA). Mean separations were determined by Tukey's test.

Table 3.1. Sugarcane clones[a] used in three controlled conditions inoculations

LCP 85-384 (HS)	HoCP 96-540 (HS)	HoCP 04-847
HoCP 85-845	L 99-226 (HS)	L 06-038
LCP 86-454	L 99-233 (R)	Ho 06-563
HoCP 91-552	L 01-283	Ho 07-613
HoCP 92-624	L 01-299 (R)	L 08-092
HoCP 92-648	L 03-371	L 09-113
Ho 95-988 (HS)	HoCP 04-838	L 09-114

[a] Commercial cultivars with known brown rust resistance reactions were included with (HS) = highly susceptible and (R) = resistant.

3.2.2 Natural infection severity ratings of clones in field nurseries

Twenty-eight clones planted in breeding program nurseries at the Louisiana State University Agricultural Center Sugar Research Station at St. Gabriel, Louisiana were rated visually for brown rust severity under natural infection conditions (Table 3.2). The ratings were recorded during the spring month of May during 2011 when *P. melanocephala* inoculum pressure was high in Louisiana. Ratings were assigned by three people based on visual

observation of symptom severity on older and younger leaves of plants in single-row field plots in three different nurseries using a 1-9 rating scale (Table 3.3). Brown rust severity ratings for 20 clones included in all three field nurseries were compared by Spearman's Rank correlation analysis using SAS version 9.4.

Table 3.2. Sugarcane clones[a] rated for brown rust resistance in three field nurseries under natural infection conditions

TucCP 77-042	HoCP 92-648	L 99-226 (HS)	HoCP 04-847
LCP 81-010	L 94-432	L 99-233 (HS)	L 06-001
LCP 85-384 (HS)	Ho 95-988 (HS)	US 01-040	Ho 06-563
HoCP 85-845	HoCP 96-540 (HS)	L 01-299 (R)	L 07-057
LCP 86-454	L 97-128	HoCP 02-618	L 07-068
HoCP 91-552	L 98-207	L 03-371	Ho 07 -613
HoCP 92-624	L 98-209	HoCP 04-838	L 08-090

[a] Commercial cultivars with known brown rust resistance reactions were included with (HS) = highly susceptible and (R) = resistant.

3.2.3 Comparison between severity ratings from controlled conditions inoculations and natural field infection ratings

Brown rust severity ratings for all three controlled conditions inoculations and natural field infection in the first and third field nurseries were compared by Spearman's rank correlation analysis using SAS version 9.4. The correlation was calculated using 16 clones in common to all five experiments.

Table 3.3. Severity rating scale for evaluation of brown rust resistance based on natural infection symptoms

Rating	Description
1	**Highly Resistant:** Little or no symptoms
2	**Resistant:** Few to moderate lesions on older leaves
3	**Moderately Resistant:** Moderate lesions on older leaves with a few lesions on young leaves
4	**Moderately Susceptible:** Moderate lesions on older leaves with necrosis and moderate lesions on some young leaves
5	**Moderately Susceptible:** Moderate to extensive lesions and necrosis on older leaves and moderate lesions on young leaves
6	**Susceptible:** Extensive lesions and necrosis on older leaves and moderate lesions with tip necrosis on young leaves
7	**Susceptible:** Extensive necrosis on older leaves and moderate to extensive lesions with tip necrosis on young leaves
8	**Highly Susceptible:** Extensive necrosis on older leaves and moderate to extensive lesions with tip necrosis on youngest leaves
9	**Highly Susceptible:** total senescence of older leaves, moderate to extensive lesions on young leaves with extensive necrosis

3.3 RESULTS

3.3.1 Resistance reactions of clones inoculated under controlled conditions

An overall analysis determined that the clone ($F = 1.22$, $P = 0.2526$), inoculation ($F = 2.88$, $P = 0.0599$), and replication (plant) ($F = 1.67$, $P = 0.1930$) effects were not significant. The clone x inoculation interaction was significant ($F = 1.54$, $P = 0.035$). Therefore, the individual experiment results were compared to determine where the variability occurred that resulted in the lack of ability to distinguish clones with different levels of resistance and whether any useful information could be obtained with controlled conditions inoculations.

Leaf area occupied by lesions ranged from 0.1 to 12.2% in inoculation 1, from 0.2 to 15.1% in inoculation 2, and from 0.1 to 3.5% in inoculation 3, and differences were detected

among clones within the inoculations (Table 3.4). Disease severity varied among clones between experiments, particularly for those exhibiting susceptibility. Resistant cultivar L 99-233 exhibited severities of 0.2, 0.6 and 0.1%, and resistant cultivar L 01-299 exhibited severities of 0.1, 0.2 and 0.1% in the first, second and third experiments, respectively, whereas the susceptible cultivars LCP 85-384, Ho 95-988, HoCP 96-540, and L 99-226 exhibited variable severities of 1.1, 1.3, and 3.5; 0.6, 15.1, and 0.8; 1.5, 0.7, and 3.0; and 0.3, 4.2, and 1.9, respectively (Table 3.4).

Severity ratings illustrated the variability for cultivars between the three inoculations, particularly for the susceptible cultivars (Table 3.4). Ratings for cultivars known to be susceptible were 8, 2, and 9; 4, 9 and 3; 9, 1, and 9; and 2, 6, and 6 for LCP 85-384, Ho 95-988, HoCP 96-540 and L 99-226, respectively. In contrast, ratings for the two resistant cultivars were 2, 1 and 1 for L 99-233 and 1 for all three experiments for L 01-299.

3.3.2 Correlation among natural field infection ratings in three nurseries

All of the 28 clones in the field nurseries exhibited some level of brown rust infection (Table 3.5). Severity ratings ranged from 3 to 8 for the first two nurseries and from 2 to 8 in the third. Ratings for the susceptible cultivars were 8, 8, and 7 for LCP 85-384; 8, 8, and 7 for HoCP 95-988; 6, 4, and 6 for HoCP 96-540; and 4, 4, and 6 for L 99-226 in the first, second, and third nurseries, respectively. Neither of the two resistant cultivars was included in all three nurseries. L 99-233 and L 01-299 had ratings of 3 and 2 and ratings of 4 and 2 in the first and third nurseries, respectively.

Table 3.4**.** Brown rust severity and resistance ratings based on severity in three controlled conditions inoculations

	Inoculation[b]								
	First		Second		Third		Average[c]		Qualitative
Clone[a]	% infection	Rating	% infection	Rating	% infection	Rating	% infection	Rating	rating[b]
LCP 85-384 (HS)	1.1 b	8	1.3 c	2	3.5 a	9	2.0	6	MS
HoCP 85-845	0.7 b	5	1.3 c	2	3.4 a	9	1.8	5	MS
LCP 86-454	4.1 b	9	0.8 c	1	0.9 bcd	3	1.9	4	MS
HoCP 91-552	0.2 b	2	1.1 c	2	0.7 cd	2	0.7	2	R
HoCP 92-624	0.3 b	2	0.5 c	1	1.8 abcd	6	0.8	3	R
HoCP 92-648	0.2 b	1	11.8 a	9	1.9 abcd	6	4.6	5	MS
Ho 95-988 (HS)	0.6 b	4	15.1 ab	9	0.8 cd	3	5.5	5	MS
HoCP 96-540 (HS)	1.5 b	9	0.7 c	1	3.0 abc	9	1.7	6	MS
L 99-226 (HS)	0.3 b	2	4.2 bc	6	1.9 abcd	6	2.1	5	MS
L 99-233 (R)	0.3 b	2	0.7 c	1	0.0 d	1	0.3	1	R
L 01-283	0.8 b	6	0.2 c	1	0.5 cd	2	0.5	3	R
L 01-299 (R)	0.1 b	1	0.2 c	1	0.1 d	1	0.1	1	R
L 03-371	0.6 b	4	0.5 c	1	2.1 abcd	6	1.1	4	MS
HoCP 04-838	0.3 b	3	3.8 bc	4	0.7 cd	2	1.6	3	R
HoCP 04-847	1.0 b	7	2.5 c	4	3.3 ab	9	2.3	7	HS
L 06-038	0.3 b	2	1.2 c	2	0.9 cd	3	0.8	2	R
Ho 06-563	0.1 b	1	2.5 c	9	0.8 cd	3	1.2	4	MS
Ho 07-613	0.9 b	7	1.2 c	2	2.0 abcd	6	1.4	5	MS
L 08-092	0.5 b	4	3.7 c	4	1.3 abcd	4	1.8	4	MS
L 09-113	0.1 b	1	0.2 c	1	0.3 d	1	0.2	1	R
L 09-114	12.2 a	9	0.9 c	2	0.8 cd	3	4.6	5	MS
Average	**1.2**	**4**	**2.6**	**3**	**1.5**	**4**	**1.8**	4	**MS**

[a] Cultivars with known brown rust resistance reactions were included with (HS) = highly susceptible and (R) = resistant.

[b] Disease severity was assessed as the percentage of leaf area occupied by brown rust lesions. Resistance ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant (R), 4 to 6 were moderately susceptible (MS), and 7 to 9 were highly susceptible (HS). Infection percentages within a column were not significantly different according to Fisher's Least Significant Difference test at $P<0.05$.

[c] Average severity infection percentages and ratings based on three inoculations.

Table 3.5**.** Brown rust severity ratings from three field nurseries for 20 clones based on natural infection severity

Clone[a]	Severity ratings from three field nurseries[b]		
	First	Second	Third
TucCP 77-042	6	3	4
LCP 81-010	7	4	6
LCP 85-384 (HS)	8	8	7
HoCP 85-845	3	3	4
LCP 86-454	4	4	4
HoCP 91-552	5	3	4
HoCP 92-624	8	NI	7
HoCP 92-648	5	6	5
L 94-432	4	4	5
Ho 95- 988 (HS)	8	8	7
HoCP 96-540 (HS)	6	4	6
L 97-128	6	4	4
L 98-207	8	8	8
L 98-209	6	4	7
L 99-226 (HS)	4	4	6
L 99-233 (R)	3	NI	2
US 01-040	4	3	4
L 01-299 (R)	4	NI	2
HoCP 02-618	4	4	5
L 03-371	4	NI	3
HoCP 04-838	4	NI	5
HoCP 04-847	5	NI	2
L 06-001	4	4	4
Ho 06-563	6	NI	6
L 07-057	4	4	6
L 07-068	4	4	4
Ho 07 -613	5	NI	6
L 08-090	6	4	6

[a] Commercial cultivars with known brown rust resistance reactions were included with (HS) = highly susceptible and (R) = resistant.

[b] Resistance ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant, 4 to 6 were moderately susceptible, and 6 to 9 were highly susceptible. NI=Not included in the nursery.

The Shapiro-Wilk test found a lack of normality in the severity ratings (Shapiro-Wilk = 0.96, $P < 0.0001$). Spearman's rank correlations for the 20 clones common to all three nurseries

indicated that only the severity ratings from the first and third field nurseries were correlated (Table 3.6).

Table 3.6**.** Spearman's rank correlation of the brown rust severity ratings based on visual symptom severity due to natural infection in three field nurseries

Nursery	First		Second		Third	
	ρ^a	*P*	ρ^a	*P*	ρ^a	*P*
First	1					
Second	-0.1692	0.4633	1			
Third	0.4710	0.0311	0.2366	0.3016	1	

[a]ρ = Spearman's rank correlation coefficient.

3.3.3 Brown rust severity rating comparisons between controlled conditions inoculations and field nurseries with natural infection

Severity ratings from controlled conditions inoculations were compared to field natural infection severity ratings for 16 clones (Table 3.7). The severity ratings showed low Spearman's rank correlations between the rankings from the three controlled conditions inoculations and two field nurseries with natural infection (Table 3.8). Ratings from the first and third controlled conditions inoculations were nearly significantly correlated ($P = 0.055$). The ratings from the first and third field nurseries from natural infection were correlated ($P = 0.007$). The average and high severity ratings of clones in controlled conditions inoculations and natural field infection were tested for correlation (Table 3.9). Clone average and high severity ratings were highly correlated for comparisons within either controlled conditions inoculations or field nurseries. In comparisons of average and high ratings between controlled conditions inoculation and natural field infection, the highest correlations were 0.41 ($P = 0.11$) between high ratings for controlled conditions inoculation and average ratings for natural field infection and 0.39 ($P = 0.13$) between average ratings for controlled conditions inoculation and natural field infection.

Table 3.7. Brown rust severity ratings from controlled conditions inoculations and field nurseries with natural infection for 16 clones

Clone[a]	Controlled conditions[b]			Natural infection[b]	
	First	Second	Third	First	Third
LCP 85-384 (HS)	8	2	9	8	7
HoCP 85-845	5	2	9	3	4
LCP 86-454	9	1	3	4	4
HoCP 91-552	2	2	2	5	4
HoCP 92-624	2	1	6	8	7
HoCP 92-648	1	9	6	5	5
Ho 95-988 (HS)	4	9	3	8	7
HoCP 96-540 (HS)	9	1	9	6	6
L 99-226 (HS)	2	6	6	4	6
L 99-233 (R)	2	1	1	3	2
L 01-283	6	1	2	8	4
L 01-299 (R)	1	1	1	4	2
HoCP 04-838	3	4	2	4	5
HoCP 04-847	7	4	9	5	2
Ho 06-563	1	9	3	6	6
Ho 07-613	7	2	6	5	5

[a] Commercial cultivars with known brown rust resistance reactions were included with (HS) =highly susceptible and (R) = resistant.
[b] Severity ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant, 4 to 6 were moderately susceptible, and 6 to 9 were highly susceptible.

Table 3.8. Spearman's rank correlations for brown rust severity ratings from three controlled conditions inoculations (CCI) and two field nurseries with natural infection (NI)

	Controlled conditions inoculation						Natural infection			
	First		Second		Third		First		Third	
Experiment	ρ^a	*P*	ρ^a	*P*	ρ^a	*P*	ρ^a	*P*	ρ^a	*P*
First CCI	1									
Second CCI	0.322	0.223	1							
Third CCI	0.488	0.055	0.209	0.43	1					
First NI	0.173	0.519	0.089	0.742	0.235	0.379	1			
Third NI	0.06	0.814	0.345	0.189	0.399	0.125	0.64	0.007	1	

[a] ρ = Spearman's rank correlation coefficient.

Table 3.9. Spearman's rank correlations for average and high brown rust severity ratings from controlled conditions inoculations (CCI) and field nurseries with natural infection (NI)

Experiment	Average CCI		Average NI		High CCI		High NI	
	ρ^a	*P*	ρ^a	*P*	ρ^a	*P*	ρ^a	*P*
Average CCI	1							
Average NI	0.393	0.132	1					
High CCI	0.815	0.0001	0.414	0.110	1			
High NI	0.325	0.2192	0.946	0.0001	0.254	0.340	1	

[a] ρ = Spearman's rank correlation coefficient.

3.4 DISCUSSION

In this study, results from inoculation of sugarcane under controlled conditions with *P. melanocephala* urediniospores could not detect consistent differences among a group of clones with variable levels of resistance to brown rust. This outcome suggests that the technique may not be useful for resistance screening. However, an evaluation of the individual experiment results for the cultivars with known resistance reactions revealed that susceptible clones exhibited variability in severity levels, whereas severities across inoculations were consistently low for resistant clones. These results suggest that accurate determination of clone resistance reactions might still be obtained by multiple inoculations.

The variable results for susceptible but not resistant clones from the controlled conditions inoculations with *P. melanocephala* are similar to what happens with resistance screening inoculations with other sugarcane pathogens, *Sporisorium scitaminea* and *Xanthomonas albilineans*, the causal agents of smut and leaf scald, respectively, that are conducted as a routine component of the cultivar selection program (Hoy unpublished). Information needed to characterize unreleased clone resistance levels is obtained by conducting multiple, annual inoculations, including cultivars with known resistance reaction, and considering average and

high clone ratings in making selection decisions. Averaging and/or taking the highest ratings for clones from multiple inoculations improved the correlation among controlled conditions inoculations and natural infection brown rust severity ratings, and the ratings were accurate for known resistant and susceptible cultivars.

Cultivated sugarcane is an interspecific hybrid with a complex aneu-polyploid genome. Sugarcane hybrids have 100-120 chromosomes with approximately 80% of the genome contributed by *S. officinarum* (2n = 80), 10–15% by *S. spontaneum* (2n = 48-124), and 5–10% from recombinations (D'Hont *et al.*, 1996; Piperidis and D'Hont, 2001). Like many traits in sugarcane, resistance to brown rust is quantitatively inherited and expressed (Comstock *et al*., 1992; D'Hont *et al*. 1996; Gonzales *et al*., 1987; Hogarth *et al*., 1993; Tai *et al*., 1981). Field resistance reactions to brown rust are known to be variable and must be acquired by repeated observations of natural infection severity over multiple seasons. Ratings from different nurseries based on natural infection severity during the same season showed variability in this study. Inoculation and symptom development under controlled conditions was evaluated to attempt to develop a method that would avoid the effects of variable environmental conditions and inoculum exposure on phenotypic expression of resistance. However, despite efforts to create uniform conditions favorable for infection and disease development (Barrera, *et al.*, 2012), erratic results were obtained for susceptible clones in different experiments. Controlled conditions inoculation and natural field infection results also were not well correlated in this study. Comparisons of average and high ratings from multiple inoculations improved the correlation with natural field infection, but the correlation was still not significant. Differential reactions between cultivars and pathogen populations from different cultivars have been demonstrated in Louisiana (Avellaneda *et al.*, 2013). The controlled conditions inoculations were

done with only urediniospores collected from cultivar Ho 95-988. The inoculum for natural infection of clones in the field nurseries would have come predominately from HoCP 96-540, a cultivar that exhibited differential reactions in comparisons with Ho 95-988. This difference could have affected the severity ratings between controlled conditions inoculation and natural field infection.

Brown rust has become the most important disease of sugarcane in Louisiana. Disease resistance has been overcome in 10 of the last 13 cultivars released from the breeding program (Hoy unpublished). Accurate characterization of resistance is needed for potential parents. However, severity ratings based on natural infection have been problematic. Multiple inoculations under controlled conditions accurately identified resistant and susceptible clones with severe infection resulting from any single inoculation indicating susceptibility. Therefore, controlled conditions inoculation has the potential to be useful in limited studies to characterize parents in a recurrent selection program and for basic studies of resistance to brown rust.

CHAPTER 4: CONCLUSIONS

- The concentration of *Puccinia melanocephala* urediniospores used for inoculation under controlled conditions strongly affects the frequency of severely infected sugarcane seedlings from any cross regardless of parental resistance levels.
- Inoculum concentrations of 1×10^4 and 1×10^5 spores/ml showed a wider distribution of frequencies of severity ratings and differences in the frequency of resistant progeny among crosses. Seedling resistance was overwhelmed at the 1×10^6 spores/ml concentration in inoculation of seedlings from crosses of all types under controlled conditions.
- Comparing infection severity assessed as ratings in the seedlings of 11 crosses, parental reaction was not a reliable determinant of the frequency of resistant progeny regardless of inoculum concentration.
- Seedling inoculation under controlled conditions to evaluate brown rust resistance will not be useful as a part of cross appraisal in the sugarcane breeding program.
- Brown rust resistance mechanisms are not fully active or can be overwhelmed in sugarcane seedlings, so screening for resistance should not be done at the seedling stage of selection.
- The severity of disease resulting from inoculation under controlled conditions did not reliably detect resistance or susceptibility in sugarcane clones with a single inoculation. However, averaged results from multiple inoculations did produce resistance ratings consistent with known cultivar resistance reactions for both resistant and susceptible cultivars.

- Sugarcane clones rated under natural field infection showed variable correlation among severity ratings assigned in different nurseries, but average ratings were accurate for cultivars with known resistance reaction.

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APPENDIX 1. Brown rust severity in three controlled conditions inoculations assessed as percentage of leaf area with lesions

	Inoculation				Inoculation		
Clone	First[a] 3/31/2011[e]	Second[b] 11/3/2011[e]	Fourth[c] 10/17/2012[e]	Clone	First[a] 3/31/2011[e]	Second[b] 11/3/2011[e]	Fourth[c] 10/17/2012[e]
LCP 81-010	0.3	ND	ND	L 05-457	0.3	ND[d]	ND
LCP 85-384	1.1	1.3	3.5	Ho 05-961	4.0	ND	ND
HoCP 85-845	0.7	1.3	3.4	HoCP 05-905	0.1	ND	ND
LCP 86-454	4.1	0.8	0.9	L 06-001	0.4	ND	0.6
HoCP 91-552	0.2	1.1	0.7	L 06-038	0.3	1.2	0.9
L 92-618	0.3	ND	0.6	L 06-040	0.2	ND	ND
HoCP 92-624	0.3	0.5	1.8	HoCP 06-537	0.3	ND	ND
HoCP 92-648	0.2	11.8	1.9	Ho 06-563	0.1	2.5	0.8
Ho 95-988	0.6	15.1	0.8	L 07-057	0.4	ND	4.6
HoCP 96-540	1.5	0.7	3.0	L 07-068	1.4	ND	5.4
L 99-226	0.3	4.2	1.9	Ho 07-613	0.9	1.2	2.0
L 99-233	0.3	0.7	0.0	Ho 07-617	0.1	10.2	ND
HoCP 00-950	0.1	0.7	ND	L 08-088	0.2	ND	ND
L 01-283	0.8	0.2	0.5	L 08-090	0.5	ND	2.0
L 01-299	0.1	0.2	0.1	L 08-092	0.5	3.7	1.3
L 03-371	0.6	0.5	2.1	L 09-105	0.2	ND	ND
HoCP 04-838	0.3	3.8	0.7	L 09-113	0.1	0.2	0.3
HoCP 04-847	1.0	2.5	3.3	L 09-114	12.2	0.9	0.8

[a] Ho 95-988 spore collection date: 6/10/2009.
[b] Ho 95-988 spore collection date: 6/9/2009.
[c] Ho 95-988 spore collection date 6/9/2010.
[d] ND= No data.
[e] Inoculation date.

APPENDIX 2. Brown rust severity ratings from three naturally infected field nurseries for all clones

Brown rust severity ratings					
First nursery		**Second nursery**		**Third nursery**	
Clone	**Rating[a]**	**Clone**	**Rating[a]**	**Clone**	**Rating[a]**
CP 48-103	5	CP 48-103	4	TuCP 77-042	4
CP 52-068	4	CP 52-068	4	US 79-010	3
CP 61-037	4	CP 72-370	3	LCP 81-010	6
CP 65-357	6	CP 74-383	4	CP 83-644	4
L 67-069	4	TuCP 77-042	3	LCP 85-384	7
CP 67 412	3	CP 77-405	3	HoCP 85-845	4
CP 70-321	3	CP 77-407	3	LCP 86-454	4
CP 72-370	4	CP 79-318	3	HoCP 91-552	4
CP 74-383	6	CP 79-348	3	HoCP 92-618	5
CP 76-331	4	LCP 81-010	4	HoCP 92-624	7
TuCP 77-042	6	LCP 82-089	6	HoCP 92-648	5
CP 77-407	4	L 83-371	3	L 94-426	5
CP 78-317	5	LCP 85-376	3	L 94-428	6
CP 78-317	5	LCP 85-384	8	L 94-432	5
US 79-010	3	CP 85-800	3	L 94-433	7
CP 79-318	4	CP 85-830	4	HoCP 95-951	5
CP 79-348	4	HoCP 85-845	3	Ho 95-988	7
US 80-004	7	LCP 86-454	4	HoCP 96-540	6
LCP 81-010	7	HoCP 89-846	7	HoCP 9651	4
LCP 81-030	3	Ho 89-889	4	L 97-128	4
LCP 82-089	6	HoCP 91-552	3	HoCP 97-609	5
LHo 83-153	5	HoCP 91-555	4	L 98-207	8
CP 83-644	4	HoCP 92-648	6	L 98-209	7
LCP 85-336	7	L 94-432	4	L 99-226	6
LCP 85-376	5	Ho 95-988	8	L 99-233	2

[a] Severity ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant, 4 to 6 were moderately susceptible, and 6 to 9 were highly susceptible.

(Appendix 2. Continued)

Brown rust severiyt ratings					
First nursery		**Second nursery**		**Third nursery**	
Clone	**Rating[a]**	**Clone**	**Rating[a]**	**Clone**	**Rating[a]**
LCP 85-384	8	HoCP 96-540	4	HoCP 00-950	4
CP 85-800	6	L 97-128	4	US 01-040	4
CP 85-830	4	L 98-207	8	L 01-135	5
HoCP 85-845	3	L 98-209	5	L 01-163	4
LCP 86-454	4	L 99-226	4	L 01-283	4
LCP 87-492	5	HoCP 00-930	5	L 01-299	2
CP 89-2143	3	US 01-040	3	HoCP 01-517	5
CP 89-831	4	L 01-281	6	HoCP 01-523	4
HoCP 89-846	8	L 01-283	4	HoCP 02-618	5
Ho 89 889	7	HoCP 02-618	4	HoCP 04-838	5
US 90-018	4	L 03-371	3	HoCP 04-847	2
HoCP 91-552	5	L 04-410	5	L 05-448	3
HoCP 91-555	5	L 05-466	3	L 05-457	3
US 92-010	4	L 05-470	7	HoCP 05-902	5
HoCP 92-618	4	L 06-001	4	Ho 05-961	3
HoCP 92-624	8	L 07-057	4	L 06-001	4
HoCP 92-648	5	L 07-068	4	L 06-040	3
L 94-424	3	L 08-090	4	L 06-138	5
L 94-426	6	L09-105	3	Ho 06-530	6
L 94-426	6	L 09-118	4	Ho 06-537	4
L 94-428	6	N 27	3	Ho 06-562	3
L 94-432	4	NCo 310	4	L 06-563	6
L 94-433	4			L 07-057	6
Ho 94 856	3			L 07-068	4
TucCP 95-25	5			Ho 07-613	5

[a] Severity ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant, 4 to 6 were moderately susceptible, and 6 to 9 were highly susceptible.

(Appendix 2. Continued)

Brown rust severity ratings					
First nursery		**Second nursery**		**Third nursery**	
Clone	**Rating[a]**	**Clone**	**Rating[a]**	**Clone**	**Rating[a]**
L 95-485	8			L 01-315	6
HoCP 95-951	7			HoCP 01-517	4
Ho 95-985	5			HoCP 01-523	5
Ho 95-988	8			HoCP 01-523	5
HoCP 96-540	6			HoCP 01-553	4
HoCP 9651	6			HoCP 01-561	4
L 97-128	6			Ho 01-564	4
L 97-137	8			US 02-089	3
HoCP 97-606	6			US 02-089	3
HoCP 97-609	4			US 02-095	4
L 98-197	5			US 02-096	6
L 98-207	8			US 02-097	6
L 98-209	6			US 02-099	4
HoCP 98-741	6			L 02-316	3
L 99-226	4			HoCP 02-610	7
L 99-233	3			HoCP 02-618	4
L 00-266	8			HoCP 02-620	5
HoCP 00-930	6			HoCP 02-623	6
HoCP 00-932	4			L 03-371	4
HoCP 00-950	5			L 04-410	7
US 01-012	5			HoCP 04-838	4
US 01-039	4			HoCP 04-847	5
US 01-040	4			L 05-448	3
L 01-283	8			L 05-451	6
L 01-299	4			L 05-451	6

[a] Severity ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant, 4 to 6 were moderately susceptible, and 6 to 9 were highly susceptible.

(Appendix 2. Continued)

Brown rust severity ratings					
First nursery		Second nursery		Third nursery	
Clone	Rating[a]	Clone	Rating[a]	Clone	Rating[a]
L 05-457	4				
L 05-460	6				
L 05-466	5				
HoCP 05-902	4				
Ho 05-961	4				
L 06-001	4				
L 06-011	5				
L 06-023	6				
L 06-038	6				
L 06-040	5				
L 06-125	8				
Ho 06-530	6				
Ho 06-537	6				
L 06-563	6				
L 07-057	4				
L 07-068	4				
Ho 07-613	5				
Ho 07-617	4				
Ho 07-617	4				
L 08-075	5				
L 08-088	3				
L 08-090	6				
L 08-092	4				
Ho 08-117	3				
Ho 08-706	5				

[a] Severity ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant, 4 to 6 were moderately susceptible, and 6 to 9 were highly susceptible.

(Appendix 2. Continued)

Brown rust severity ratings					
First nursery		Second nursery		Third nursery	
Clone	Rating[a]	Clone	Rating[a]	Clone	Rating[a]
Ho 08-709	3				
Ho 08-711	5				
HoL 08-723	4				
HoCP 08-726	4				
Ho 08-730	5				
Ho 08-9616	4				
Ho 08-9617	4				
Ho 08-9618	5				
L 09-099	5				
L 09-102	5				
L 09-107	5				
L 09-108	4				
L 09-112	3				
L 09-114	5				
L 09-117	6				
L 09-118	4				
L 09-121	4				
L 09-123	4				
L 09-129	5				
L 09-131	7				
HoCP 09-800	4				
HoCP 09-803	3				
HoCP 09-804	3				
HoCP 09-810	6				
HoCP 09-814	4				

[a] Severity ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant, 4 to 6 were moderately susceptible, and 6 to 9 were highly susceptible.

(Appendix 2. Continued)

Brown rust severity ratings					
First nursery		**Second nursery**		**Third nursery**	
Clone	**Rating[a]**	**Clone**	**Rating[a]**	**Clone**	**Rating[a]**
Ho 09-822	3				
Ho 09-824	4				
Ho 09-825	4				
Ho 09-827	3				
Ho 09-831	5				
Ho 09-832	3				
Ho 09-840	5				
Ho 09-841	3				
HoCP 09-846	6				
N 27	3				

[a] Severity ratings were assigned on a 1–9 scale in which ratings of 1 to 3 were resistant, 4 to 6 were moderately susceptible, and 6 to 9 were highly susceptible.

VITA

Mavir Carolina Avellaneda Barbosa was born in Bogotá, Colombia in 1981. In 1997, she started her bachelor studies in Industrial Microbiology in the Pontificia Universidad Javeriana in Bogotá, Colombia. She began her research career as an intern student in the Colombian Sugarcane Research Center-CENICANA under the mentoring of Dr. Jorge Victoria. Her project of research during her internship was supported with the National Phytopathological Award from the Colombian Plant Pathology Association ASCOLFI. After her graduation in 2002, she was a junior researcher in CENICANA sponsored by COLCIENCIAS (Administrative Department of Science, Technology and Innovation of Colombia). During seven years, she was involved in different projects related with sugarcane viral diseases.

In January 2011, she joined Dr. Jeff Hoy's lab and started to work on the screening for brown rust using controlled conditions methods. During her time in graduate school she was awarded with LSU Graduate School Travel Award, LACA (Louisiana Agricultural Consultant Association) and ASSCT (American Society of Sugarcane Technologist) fellowships. In 2014, she was invited to join the Omicron Delta Kappa Honor Society and has served as treasurer of the PPCP-Graduate Student Association since June 2013. She will receive the Master of Science degree in Plant Health in August, 2014.